Chronicles of the travelling gut

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Chronicles of the travelling gut:

How travel influences long-term microbiota-associated health and antimicrobial resistance dynamics

Jiyang Chan

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Chronicles of the Travelling Gut:

How Travel Influences Long-Term Microbiota-Associated Health and Antimicrobial Resistance Dynamics

Proefschrift

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CHAPTER 1

General Introduction

Travel has become an integral part of modern life. The ease of global mobility and the rapid expansion of international connections have made intercontinental travel more accessible than ever before. Following the sharp decline caused by the COVID-19 pandemic, the world has witnessed a strong resurgence in travel for both leisure and business purposes[1,2]. As travel volumes continue to rise, the health of travellers has once again become a key focus for clinicians, public health professionals, and travellers themselves. Each journey carries its own narrative, shaped by exposure to new environments, food and water sources, healthcare systems, and microbial ecologies. Although most journeys unfold without medical complications, travel can expose individuals to a wide range of infectious diseases and other health risks. Among the most important and interrelated of these are travellers' diarrhoea (TD), the acquisition and dissemination of antimicrobial resistance (AMR), and post-travel sequelae arising following TD.

Travellers' Diarrhoea

TD remains the most commonly occurring travel-related illness. Depending on the destination, season, and travel conditions, attack rates typically range between 10% and 40%, with the highest incidence observed among travellers visiting high-risk regions such as the Middle East, South and Southeast Asia, and Latin America and the Caribbean[3,4]. Symptoms most commonly occur during the first week of travel, reflecting early exposure to enteric pathogens. Common bacterial causes include pathogenic *Escherichia coli*, *Campylobacter*, *Shigella*, and *Salmonella,* while viral and parasitic agents also contribute and vary by geography[5,6]. Although most episodes of TD are self-limiting, more severe cases or those accompanied by dysentery may require antibiotics use. More importantly, TD can be caused by antimicrobial resistant enteropathogens, resulting in AMR acquisition and potential dissemination, as well as serve as a gateway exposure that predisposes individuals to longer-term sequelae, including the development of post-infection irritable bowel syndrome (PI-IBS).

Post-infection irritable bowel syndrome

Meta-analyses indicate that individuals who experience infectious enteritis have more than a fourfold increased risk of developing irritable bowel syndrome (IBS) compared with uninfected controls, with the overall prevalence of post-infection IBS (PI-IBS) ranging from approximately 11% to 15% following an episode of acute gastroenteritis[7,8]. This condition is characterized by recurrent abdominal pain, bloating, urgency and altered bowel habits in individuals with no prior history of gastrointestinal disorders [9]. The likelihood of developing PI-IBS is influenced by multiple factors, such as the causative pathogen, the intensity and duration of the acute episode, and psychological stressors, with females demonstrating a higher predisposition than males[7,8]. Mechanistically, PI-IBS is thought to involve persistent low-grade mucosal inflammation, increased intestinal permeability, altered gut–brain axis signalling, and microbiome dysbiosis[9,10]. However, its exact pathophysiology remains incompletely understood and continues to be an active area of investigation. Given that TD represents a common manifestation of infectious gastroenteritis during travel, it follows that a subset of affected travellers may subsequently develop PI-IBS[11]. Investigating this phenomenon in travellers offers a valuable opportunity to elucidate the mechanisms linking acute enteric infection to chronic gastrointestinal disorders. Travel provides a well-defined and time-bound natural model in which both exposure and outcome can be closely monitored. Insights gained from such research can enhance understanding of PI-IBS pathophysiology and inform the development of strategies to prevent or mitigate post-infection complications in travellers and the wider population.

Travel and the spread of antimicrobial resistance

Simultaneously, intercontinental travel has emerged as a key factor influencing the global spread of AMR, which represents an escalating threat to public health worldwide. According to the World Health Organization Global Antibiotic Resistance Surveillance Report 2025, between 2018 and 2023, antibiotic resistance increased in more than 40% of the monitored pathogen–antibiotic combinations, with an average annual rise of 5–15%[12]. A systematic analysis report estimated that drug-resistant infections were directly responsible for 1.27 million deaths and associated with nearly 4.95 million deaths worldwide in 2019[13]. The frequent movement of people between regions with differing antibiotic use practices and microbial ecologies facilitates the exchange of resistant microorganisms and genes, underscoring the role of travel in the intercontinental dissemination of AMR. Around 30% of travellers return with AMR bacteria[14]. Among these, Enterobacterales producing extended-spectrum β-lactamases (ESBL-PE) are the most frequently reported[15]. Numerous prospective cohort studies and meta-analyses have documented high post-travel colonization rates with ESBL-PE, particularly after travel to South and Southeast Asia, Africa, and Latin America, with reported acquisition rates ranging from 20% to 70% depending on destination and behaviour[16-18]. Apart from beta-lactam resistance, intercontinental travel also contributes to the global spread of carbapenemase-producing Enterobacterales (CPE), plasmid-mediated colistin resistance genes (mcr), and fluoroquinolone- or macrolide-resistance, albeit at lower frequencies[19-22]. Risk factors consistently associated with AMR acquisition include travel to regions with poor sanitation and high antimicrobial consumption, as well as antibiotic use during travel, particularly for TD, which substantially increases both the likelihood and duration of colonization[22].

Beyond resistance observed in clinically relevant (opportunistic) pathogens, the broader collection of resistance genes within microbial ecosystems, known as the resistome, is of interest. Because resistance determinants can disseminate between bacteria through horizontal gene transfer (HGT), all resistance elements within these ecosystems are potentially relevant to human health, as incoming bacteria harbouring AMR genes can be transferred through HGT to the indigenous microbial communities, including opportunistic pathogens[23-25]. Investigating the AMR gene repertoire, rather than clinical isolates alone, therefore provides a more comprehensive understanding of how AMR emerges and spreads. Returning travellers may act as transient reservoirs and vectors of resistant microorganisms, contributing to their dissemination within households, communities, and healthcare settings[14,15,26,27].

Gut Microbiota at the Crossroads of Travel, AMR, and PI-IBS

Taken together, this highlights the interconnected nature of AMR acquisition, travel, and human health, emphasizing the gut as a central interface in these processes. The gut microbiota constitutes a dense and dynamic microbial ecosystem that responds rapidly to external exposures such as dietary change, environmental factors, antibiotic use, and travel which we will highlight in **Chapter 2**. Such microbiome perturbations can facilitate the acquisition and dissemination of AMR genes while also influencing host physiology and susceptibility to diseases and potential long-term sequela. Longitudinal studies have shown that travel induces measurable shifts in gut microbiota composition, regularly accompanied by the acquisition of resistant bacteria and genes[26,28,29]. Although overall diversity often remains stable, episodes of travellers' diarrhoea are associated with marked community disruption and a temporary rise in AMR gene abundance that can persist for weeks[30]. Yet, despite these insights, studies integrating gut microbiota dynamics with AMR acquisition during travel remain scarce. The timing, persistence, and ecological drivers of these changes are still not fully understood.

Similarly, disruptions in gut microbial homeostasis following enteric infection have been implicated in the development of PI-IBS[31-33]. Jalanka-Tuovinen *et al.* first characterized microbial differences between healthy individuals and PI-IBS patients, reporting an increased abundance of several members of the phylum *Bacteroidetes* (now *Bacteroidota*) and a depletion of uncultured *Clostridia* in PI-IBS[31]. More recently, Edwinson *et al.* demonstrated that PI-IBS patients exhibit reduced *Alistipes* abundance and diminished microbial β-glucuronidase activity, leading to impaired bilirubin deconjugation and elevated intestinal proteolytic activity that may compromise barrier integrity[32]. However, the microbial mechanisms linking acute infection to long-term functional disturbances remain poorly defined, largely due to the limited temporal resolution of existing studies, which have predominantly investigated PI-IBS after symptom onset and thus provide little insight into its early development[31-33]. Understanding gut microbiota dynamics in the context of both AMR acquisition and PI-IBS development therefore represents an important yet understudied opportunity to elucidate how transient ecological disturbances during travel can lead to divergent health outcomes with microbial resistance on one hand, and chronic gastrointestinal dysfunction on the other. This leads us to current gaps in knowledge that needs to be addressed:

- **Longitudinal gaps**: Most existing studies assess only pre- and post-travel samples, overlooking the critical window "during-travel" when microbial and AMR gene repertoire changes occur. Moreover, the temporal evolution of post-travel sequelae such as PI-IBS remains poorly defined, as current studies are retrospective or cross-sectional. Prospective data that begin before disorder onset and extend sufficiently post-travel are virtually non-existent, limiting our understanding of how transient disturbances transition to chronic outcomes.
- **Frequency gaps**: High-frequency sampling across the full travel arc, from departure through return, is uncommon but essential to capture short-lived microbial fluctuations, acquisition events, and recovery trajectories. Without dense temporal resolution, key moments in microbiota and AMR genes dynamics remain unresolved.

- **Exposure context gap**: Limited data exist on how lifestyle, environmental, and travel-related exposures influence microbiome and AMR gene repertoire changes. Incorporating these determinants is essential to understand inter-individual variability and identify modifiable risk factors for adverse outcomes.
- **Diagnostics and prediction gaps**: There is currently a lack of reliable microbial biomarkers to identify travellers at risk of PI-IBS following an episode of gastroenteritis as well as to diagnose this disorder at an early stage. While factors such as destination, behaviour, and demographics account for part of the risk, explainable models integrating microbiota and host data are still lacking.

Aims and scope of this thesis

This thesis aims to characterize the impact of intercontinental travel on the human gut microbiome and AMR gene collection, and to elucidate their roles in post-infection sequelae such as PI-IBS. The research integrates prospective cohort studies, and temporal sampling to investigate microbiota dynamics, AMR gene acquisition, and clinical outcomes among intercontinental travellers

In **Chapter 2**, we synthesized current knowledge on the travel-associated gut microbiome and AMR gene profile, summarizing how intercontinental mobility can shape microbial diversity, function, and antimicrobial resistance acquisition. This review highlights mechanisms of disruption, recovery, and transmission, and identifies current knowledge gaps that inform future studies.

In **Chapter 3**, we assessed the incidence and risk factors of PI-IBS following intercontinental travel through a prospective, multicentre cohort study. We aimed to establish the burden of PI-IBS in travellers and to delineate demographic and behavioural determinants associated with its development through traditional statistics approach and machine learning methods.

The project described in **Chapter 4** aimed to elucidate the relationship between gut microbiota composition in intercontinental travellers and their environmental, lifestyle, and travel-related exposures, with post-infection irritable bowel syndrome (PI-IBS) examined within a nested case–control subset of the overall travel cohort. We sought to determine how these exposures influence the gut microbiota and whether microbiota alterations precede or result from infection, thereby advancing our understanding of PI-IBS pathogenesis.

In **Chapter 5**, we quantified the acquisition of AMR genes among Dutch intercontinental travellers. This chapter reports the influence of destination and travel-related exposures on AMR genes dynamics and identifies behavioural and environmental determinants of AMR acquisition.

Chapter 6 defines the fine-scale temporal sequence of microbiota and resistome alterations using high-frequency (daily) longitudinal sampling during travel. This approach captures the earliest microbial shifts associated with intercontinental travel, offering insight into subject-specific and population-level AMR and microbiota dynamics before, during and after travel.

In **Chapter 7**, I reflect on the key findings from this thesis, placing them in the broader context of travel medicine, microbiome research, and antimicrobial resistance. This chapter also outlines the methodological and conceptual implications of the work and proposes directions for future research in traveller cohorts, including strategies to mitigate microbiome disruption and monitoring AMR.

Chapter 8 is dedicated to the impact of our research, detailing its scientific contribution, relevance for society and public health, and the dissemination of results to academic, clinical, and non-academic audiences.

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CHAPTER 2

The travellers' gut microbiome: environmental perturbation, adaptation, and antimicrobial resistance on a global scale

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Manuscript in preparation

CHAPTER 3

Post-infection irritable bowel syndrome after intercontinental travel: a prospective, multicentre study

Based on:
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Jarne van Hattem, Damian C. Melles, Menno C. de Jong,
Constance D. Schultsz, Perry J.J. van Genderen,
John Penders & COMBAT-study consortium

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Abstract

Post-infection irritable bowel syndrome (PI-IBS) can emerge after acute infectious gastroenteritis, particularly traveller's diarrhoea (TD), yet its underlying mechanisms and modifiable risk factors remain unclear due to limitations in prior cross-sectional research. This study aimed to determine the incidence and predictors of PI-IBS in a large, prospective cohort of intercontinental travellers by analysing data from the COMBAT study, which included 2,001 Dutch travellers between 2012 and 2013. Participants completed questionnaires before travel, immediately after returning, and at 1-, 3-, 6-, and 12-months post-travel; those who experienced TD and had no prior IBS were considered at risk. PI-IBS was defined according to Rome III criteria as the presence of IBS symptoms for at least six months following TD. Among 539 at-risk travellers, 65 developed PI-IBS, resulting in an incidence of 12.1% (95% CI: 8.3–13.9), with the highest incidence reported in travellers to Southern Asia (26.4%). Independent predictors of PI-IBS included stomach cramps, chronic illness, nausea, weight loss, non-excellent self-rated health, vegan/vegetarian diets, antibiotic use, and antacid use. Machine learning models, particularly neural networks, showed good predictive performance (AUROC 0.77) and identified stomach cramps, nausea, and anxiety as top predictors. This study underscores a substantial incidence of PI-IBS following TD and highlights the predictive value of health and behavioural factors during travel, offering new insights for prevention and risk stratification.

Introduction

Post-infection irritable bowel syndrome (PI-IBS) is a gastrointestinal disorder that can arise following an episode of acute infectious gastroenteritis and is often marked by persistent abdominal discomfort and altered bowel habits.[1] Irritable bowel syndrome (IBS) can have implications for patient quality of life, productivity, and healthcare costs[2,3]. Despite increasing epidemiological understanding, its pathophysiology remains elusive. Understanding and preventing PI-IBS requires identifying modifiable risk factors and individuals at risk. An accurate evaluation of predictors of PI-IBS would therefore aid in devising preventive measures and improve further research into mechanistic aspects of (PI-)IBS development.

A comprehensive meta-analysis by Klem et al. showed several risk factors that have been associated with the development of PI-IBS, including the severity and duration of the initial infection, female gender, younger age, psychological comorbidities such as anxiety and depression, and the presence of certain pathogens like *Giardia duodenalis*[4]. However, the cross-sectional design of most of these studies prevents the estimation of the incidence of PI-IBS. Moreover, most studies inconsistently reported confounding adjustments, restricting the inference that can be drawn from their results[4]. Given the strong association with traveller's diarrhoea (TD), several previous cohorts have evaluated PI-IBS development amongst travellers[5]. However, these studies investigated a limited number of predictors prior to or during the onset of PI-IBS. Moreover, many of these studies have used traditional regression-type models to investigate predictors associated with PI-IBS. Conventional statistical methods may not take into account the complex structure of unknown interactions and non-linear associations and therefore combining machine learning modelling with standard approaches may benefit predictors analysis[6].

To address the gaps, we conducted a longitudinal analysis within the world's largest traveller cohort to assess the incidence of new-onset PI-IBS after TD, and to identify a broad set of potential predictive factors, both before, during and after travel, using logistic regression and machine-learning approaches.

Methods

Study population and design

As part of the COMBAT study, 2001 travellers were recruited from outpatient clinics in the Netherlands between 2012 and 2013[7]. Questionnaires, including ROME III questions for functional gastrointestinal disorders[8], were filled before travel, immediately after return and at 1,3,6 and 12 months after return. Ethical approval was granted by the Medical Ethical Committee of Maastricht University Medical Centre (METC-12-4-093). More details on the study design are published elsewhere[7].

Study measures

For the present study, we focussed on travellers at-risk for PI-IBS development, which was defined as travellers without IBS symptoms prior to travel and who experienced diarrhoea during travel (TD). TD was defined as ≥3 loose stools per day for at least 1 day. At risk travellers with new onset IBS following gastroenteritis were classified as PI IBS cases if they met Rome III criteria, defined as recurrent abdominal pain or discomfort occurring at least 3 days per month for 3 months, associated with at least two of improvement with defecation, change in stool frequency, or change in stool form, with symptoms persisting for at least 6 months after travel.

Additional questionnaire data were collected to obtain information on potential risk factors for PI IBS development after travel, including demographics, health status, medication use, and behaviours before and during travel. Age and BMI were calculated using baseline age, weight, and height of participants recorded directly prior to arrival destination.

Statistical analysis

Logistic regression models were used to identify predictors of PI-IBS development. Variables were tested for multi-collinearity through variance inflation factor (VIF) and correlation matrices. In order to mitigate unstable estimates and large standard errors, a selection method based on the approach proposed by Hosmer and Lemeshow was applied to identify predictors associated with the development of PI-IBS after travel[9]. The process began by performing univariable analysis for each individual predictor as independent variable and PI-IBS development as dependent variable. Variables were then selected based on a cut-off point of $p<0.25$(Wald test). Using the more conventional threshold of 0.05 may fail in identifying factors that are known to be important[10]. Variables were tested for multi-collinearity before being entered into the multivariable analysis and machine learning process. In the multivariable analysis, statistically insignificant variables ($p>0.05$, Wald test) were removed one at a time. In case the removal of a variable resulted in a change in one of the remaining parameters estimates with >20% compared to full model, the variable was retained as it suggests confounding. The final model only included significant variables ($p<0.05$) and confounders. Results were presented as odds ratios (OR) and 95% confidence intervals (95%CI). Statistical analyses were performed using IBM SPSS Statistics (version 27).

Random forest (RF), neural networks (NN), and Support Vector Machine with Radial Basis Function kernel (SVM-RBF) were used to predict PI-IBS onset in at-risk travellers. In the machine learning process, only complete cases were considered and 5-fold cross-validation was repeated 10 times for performance assessment. This entails dividing the data into subsets, training the data on one subset, and employing the other subset to assess the model's performance. To account for variability, multiple iterations of cross-validation was performed utilizing distinct subsets derived from the same dataset. The validation outcomes from these iterations were next aggregated to derive an approximation of the model's predictive capability. Within the repeated cross-validation framework, hyperparameter tuning was performed using Grid Search combined with down sampling to achieve equal class distributions. Area under the receiver operating characteristic (AUROC) was used as an evaluation metric and 95% CI was calculated using the DeLong method[11]. The machine learning process was performed in R (v4.1.2) using Caret (v6.0-94) and recipes (v1.0.5) packages, and receiver operating characteristic (ROC) curve plots were made with pROC (v1.18.0). RF, NN and SVM-RBF models were made using respectively ranger v0.14.1, nnet v7.3-18 and kernlab v0.9-32 packages.

Mapping incidence

Incidence proportion map was made using packages rnaturalearth v0.3.2 and sf v1.0-12.

Results

A total of 539 travellers were at risk of acquiring PI-IBS of whom 65 developed PI-IBS after travel (Figure 1A and supplementary Table 1), resulting in an incidence of 12.1% (95%CI 8.3-13.9). The highest incidence was observed in travellers to Southern Asia, among whom 14 out of 53 at-risk travellers developed PI-IBS (26.4%, 95%CI 12.2–27.5, Figure 1B, Supplementary Table 1), followed by travellers visiting multiple sub regions within Africa (20.0%, 95% CI 8.3-30.6) and South-America (16.7%, 95% CI 7.3-20.9). A total of 33 out of 960 (3.4%) travellers developed IBS without TD after travel whilst 65 out of 539 at-risk travellers developed PI-IBS (relative risk: 3.51, 95%CI 2.34–5.26, $P < 0.001$). In addition, the majority (78.7%) retained PI-IBS symptoms up to 12 months after travel.

We next performed univariable logistic regression analyses, followed by multivariable logistic regression, to identify determinants for PI-IBS development in travellers. In the multivariable regression (Supplementary Table 2), experiencing stomach cramps during travel was the strongest independent predictor for PI-IBS acquisition ($OR_{adjusted}$ 5.92, 95%CI 2.17-16.12). This was followed by predictors related to health and diet. Having a pre-existing chronic illness (2.35, 95% CI 1.08-5.13), following a specific diet (vegetarian/vegan: 3.49, 95% CI 1.13-10.73; other diet: 5.69, 95% CI 1.56-20.82), reporting less than excellent general health during travel (good: 5.09, 95% CI 1.59-16.33; reasonable, mediocre or bad: 5.47, 95% CI 1.23-24.33), experiencing nausea during travel (2.25, 95% CI 1.08-4.69), sustained weight loss during travel (2.24, 95% CI 1.07-4.70), and taking antibiotics during travel (2.96, 95% CI 1.14-7.66) were all positively associated with PI-IBS acquisition.

Thereafter, we applied three machine learning algorithms (RF, NN and SVM-RBF) to predict PI-IBS development and restricted the input to predictors with $p<0.25$ in the univariable analyses and complete cases (N = 510 of which 61 developed PI-IBS). All three methods performed similarly with area under the receiver operator curves (AUROCs) between 0.74 and 0.77 (Supplementary Figure 1). We subsequently chose a NN model as the best performing algorithm based on the slightly higher overall AUROC value as compared to the other models. The final NN model (parameters: weight decay = 4; hidden layer size = 5) was able to classify new onset PI-IBS with an overall AUROC value of 0.767 (95% CI 0.75-0.786), median sensitivity of 0.66 (95% CI 0.63-0.70, bootstrap method) and specificity of 0.73 (95% CI 0.72- 0.74, bootstrap method). Like the multivariable regression, predictors related to health issues during travel contributed most to the prediction of developing PI-IBS (Figure 1C). Results were very similar to other evaluated ML techniques (data not shown).

Amongst the factors in the multivariable logistic regression; antibiotic use, nausea, stomach cramp and weight loss during travel were also found to be important predictors for PI-IBS development after TD in the NN-model. Along with TD, this may represent a more severe infection and subsequently a higher risk of developing PI-IBS. Other indicators of more severe infection were not statistically significantly increased (fever 7.9% vs 15.6%, $P = 0.10$) or reported too rarely to be included in the analyses (dysentery reported by 10 'at-risk' travellers).

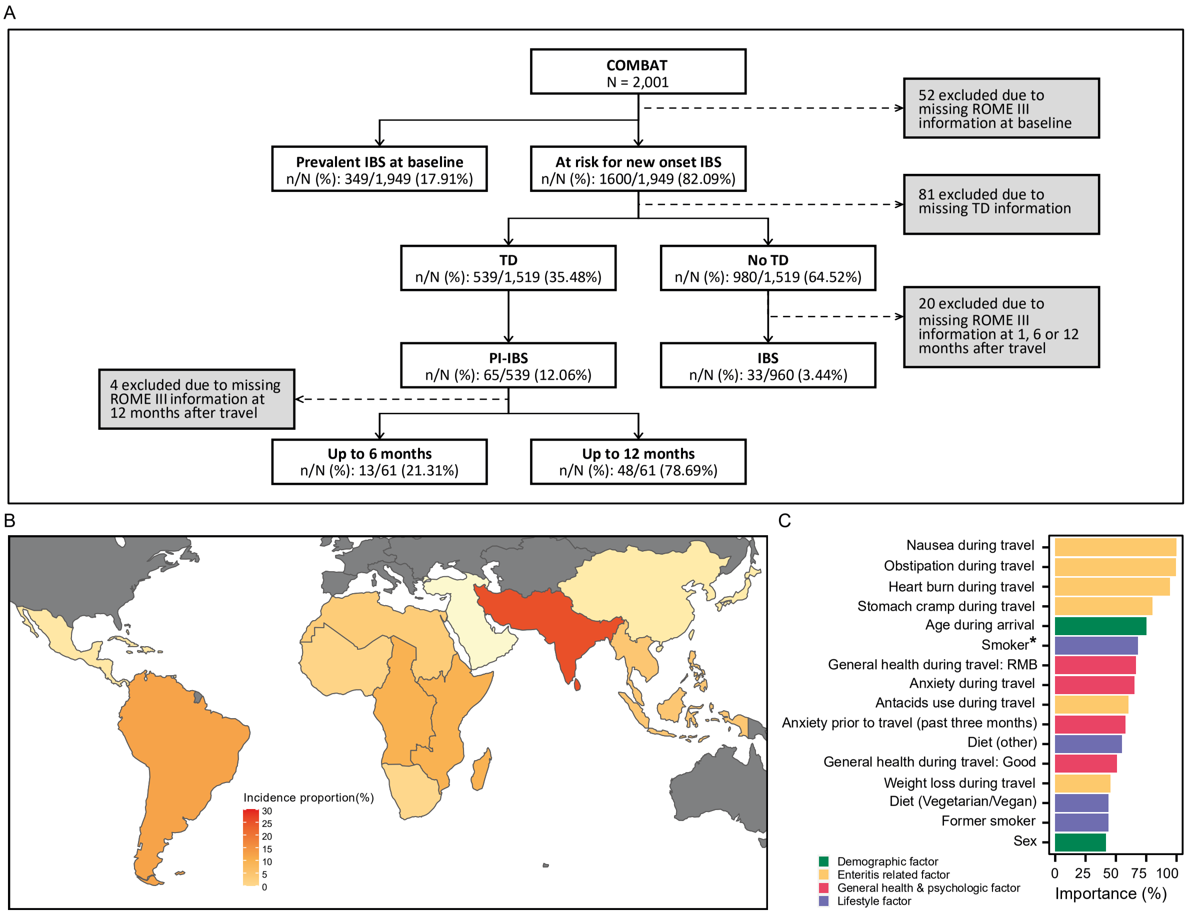
A
COMBAT
N = 2,001
52 excluded due to missing ROME III information at baseline
Prevalent IBS at baseline
n/N (%): 349/1,949 (17.91%)
At risk for new onset IBS
n/N (%): 1600/1,949 (82.09%)
81 excluded due to missing TD information
TD
n/N (%): 539/1,519 (35.48%)
No TD
n/N (%): 980/1,519 (64.52%)
20 excluded due to missing ROME III information at 1, 6 or 12 months after travel
PI-IBS
n/N (%): 65/539 (12.06%)
IBS
n/N (%): 33/960 (3.44%)
4 excluded due to missing ROME III information at 12 months after travel
Up to 6 months
n/N (%): 13/61 (21.31%)
Up to 12 months
n/N (%): 48/61 (78.69%)
B
Incidence proportion(%)
30
25
20
15
10
5
0
C
Nausea during travel
Obstipation during travel
Heart burn during travel
Stomach cramp during travel
Age during arrival
Smoker*
General health during travel: RMB
Anxiety during travel
Antacids use during travel
Anxiety prior to travel (past three months)
Diet (other)
General health during travel: Good
Weight loss during travel
Diet (Vegetarian/Vegan)
Former smoker
Sex
Demographic factor
Enteritis related factor
General health & psychologic factor
Lifestyle factor
0
25
50
75
100
Importance (%)

Figure 1. (A) Flowchart of study (B) Incidence proportion (%) of travellers that developed PI-IBS after TD per United Nations sub region. (C) Top 16 feature contribution for the neural network model displayed as importance in percentages from 0% to 100%. A neural network model is a machine learning method consisting of interconnected artificial neurons organized in layers that adjusts weights between neurons during model training to minimize errors. The importance of input variables on the network's outcomes was calculated by analysing the weights and connections associated with each variable, as well as the changes in performance when these variables are altered or removed. RMB = reasonable, mediocre, or bad: *inverse association with PI-IBS development.

Discussion

This study aimed to prospectively assess the incidence of post-infection irritable bowel syndrome (PI-IBS) after traveller's diarrhoea (TD) and to identify predictors contributing to its development. We found that the incidence of PI-IBS following TD was substantial, and that risk varied markedly by travel destination. Notably, travellers to Southern Asia exhibited the highest incidence, with more than one-quarter of at-risk individuals developing PI-IBS.

Unique to our study was the ability to calculate incidence proportions according to travel destination. Geographic differences in PI-IBS incidence are likely influenced by variation in the predominant causative agents of gastroenteritis. Enterotoxigenic *Escherichia coli* is the predominant pathogen causing TD in South America, Africa and Southern Asia whilst *Campylobacter* species is more common in Southern and South-East Asia[4,12]. These pathogen profiles may partially explain the regional differences observed. The overall incidence in our cohort aligns with the estimated point prevalence of 11.5% reported in a large meta-analysis of PI-IBS after infectious enteritis[4]. However, the incidence was more than twice as high compared to another meta-analysis (pooled incidence of 5.4%) specifically focussing on PI-IBS after TD[5]. This difference could be attributed to the limited size and high variability in study design between studies included in this latter meta-analysis.

By integrating a broad array of variables before, during and after gastroenteritis onset, our study underscores the value of a comprehensive approach to identifying PI-IBS risk factors. Several associations identified here, such as antibiotic use and weight loss, are consistent with previous literature[4,13]. In contrast, factors previously associated with PI-IBS, such as sex and anxiety, did not retain significance in the multivariable model. This attenuation may result from controlling for confounders or including variables along causal pathways. For example, stomach cramps may reflect infection severity but could also be influenced by sex-related hormonal or anxiety-related differences in nociception. Nevertheless, anxiety before and during travel emerged as important predictors in the neural network model, which is compatible with known links between anxiety, somatization, and increased susceptibility to infectious gastroenteritis through stress-related impairment of barrier or immune function[14,15].

We also observed a potential protective association of smoking with PI-IBS development. Although, smoking is recognized to improve ulcerative colitis, presumably through anti-inflammatory effects mediated by compounds in cigarette smoke[16], the mechanisms underlying any protective effect in PI-IBS remain yet unknown.

Our prediction model additionally identified antacid use during travel as an important predictor of PI-IBS development. Antacids potentially enhance gastroenteritis severity by reducing stomach acid, a crucial barrier against infection, thereby increasing the risk of developing PI-IBS. This hypothesis is supported by recent murine data showing that antacid pre-treatment expanded the founding population size of enteropathogens in the gut, heightened infection susceptibility, and exacerbated morbidity[17]. These results highlight the potential of combining machine learning models with more traditional epidemiologic statistical methods to uncover risk factors for complex, multifactorial conditions such as PI-IBS. This study also has limitations. The use of Rome III criteria, rather than Rome IV, to assess IBS might be considered as a constraint. However, Rome IV criteria are more restrictive and might underestimate the true incidence in epidemiological surveys[18]. Additionally, external validation of our machine learning models was not possible due to the unique nature of the cohort, which may limit generalisability and increase the risk of overfitting.

Despite these potential limitations, this was, to our knowledge, the first study incorporating a comprehensive collection of predictors in prospectively investigating PI-IBS development after TD. Our findings demonstrate that PI-IBS is a frequent sequela of TD and provide important insights into its multifactorial aetiology, supporting efforts toward targeted prevention and management strategies.

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Author contributions

JC performed all data analysis and data interpretation. MW supported the data analysis. NvB, PJJvG and JP contributed to the data interpretation and provided guidance on the data analysis. MSA, JMvH, DCM, MDdJ, CS, PJJvG and JP conceived the study design and acquired funding. JC drafted the manuscript with critical review by NvB, PJJvG and JP. All the authors read and contributed on the final drafting.

Conflict of Interest/Disclosure

The authors have declared no conflicts of interest.

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Supplementary materials

Supplementary Table 1. Incidence proportion for PI-IBS development in Dutch intercontinental travellers after traveller's diarrhoea by sub region.

	Number of travellers At risk (n=539)	Number of travellers who acquired PI-IBS (n=65)	PI-IBS incidence proportion (95% CI)‡
Central and Eastern Africa	68 (12.6%)	10 (15.4%)	14.7 (6.9-18.4)
Northern Africa	30 (5.6%)	3 (4.6%)	10.0 (3.8-17.6)
Southern Africa	26 (4.8%)	2 (3.1%)	7.7 (3.0-16.5)
Western Africa	36 (6.7%)	3 (4.6%)	8.3 (3.1-15.1)
Multiple sub regions in Africa	15 (2.8%)	3 (4.6%)	20.0 (8.3-30.6)
Caribbean and Central America	21 (3.9%)	1 (1.5%)	4.8 (2.4-15.5)
South America	48 (8.9%)	8 (12.3%)	16.7 (7.3-20.9)
Multiple sub regions in America	8 (1.5%)	0 (0.0%)	0.00
Eastern Asia	24 (4.5%)	1 (1.5%)	4.2 (1.9-13.8)
Southern Asia	53 (9.8%)	14 (21.5%)	26.4 (12.2-27.5)
South eastern Asia	176 (32.7%)	19 (29.2%)	10.8 (6.1-13.4)
Western Asia	5 (0.9%)	0 (0.0%)	0.00
Multiple sub regions in Asia	19 (3.5%)	1 (1.5%)	5.3 (2.8-16.9)
Multiple regions	10 (1.9%)	0 (0.0%)	0.00
Total	539 (100.0%)	65 (100.0%)	12.1 (8.3-13.9)

‡Based on binomial distribution (Wilson's score interval).

Supplementary Table 2. Logistic regression analysis on potential predictors for PI-IBS acquisition among at risk travellers (n=539)

	All travellers at risk (n=539)*	**Travellers who developed PI-IBS** (n=65, n/N%)**	**OR (95% CI)**	**P-value**	**Adjusted OR (95% CI)**	**Adjusted p-value**
Median age during arrival at destination [IQR]	52.44 [33.28:60.64]	42.61 [27.43:57.14]	0.972 (0.955-0.988)	<0.001	0.988 (0.961-1.015)	0.379
Median BMI in kg/square meters [IQR]	24.69 [22.60:27.13]	24.09 [22.61:26.15]	0.95 (0.877-1.03)	0.233	0.963 (0.874-1.06)	0.439
Sex						
Male	269 (49.9%)	20/269 (7.4%)	1			
Female	270 (50.1%)	45/270 (16.7%)	2.490 (1.427-4.345)	0.001	1.473 (0.714-3.038)	0.295
Education level						
No education, elementary school or prevocational secondary education	64 (11.9%)	7/64 (10.9%)	1			
Vocational secondary education, senior general secondary education or education up to university	125 (23.2%)	15/125 (12.0%)	1.11 (0.428-2.878)	0.829		
Higher professional education	189 (35.1%)	19/189 (10.1%)	0.910 (0.364-2.277)	0.84		
Academic (university) education	161 (29.9%)	24/161 (14.9%)	1.426 (0.582-3.497)	0.438		
Drinking alcohol						
No	42 (7.8%)	8/42 (19.0%)	1			
Yes	497 (92.2%)	57/497 (11.5%)	0.551 (0.243-1.248)	0.153		
Smoking situation prior to travel						

	All travellers at risk (n=539)*	**Travellers who developed PI-IBS** (n=65, n/N%)**	**OR (95% CI)**	**P-value**	**Adjusted OR (95% CI)**	**Adjusted p-value**
No	228 (42.5%)	31/228 (13.6%)	1			
Former smoker	196 (36.6%)	24/196 (12.2%)	0.887 (0.501-1.569)	0.68	1.17 (0.532-2.574)	0.696
Yes	112 (20.9%)	9/112 (8.0%)	0.555 (0.255-1.211)	0.139	0.28 (0.099-0.793)	0.017
Region visited						
America	77 (14.3%)	9/77 (11.7%)	1			
Africa	175 (32.5%)	21/175 (12.0%)	1.030 (0.449-2.366)	0.944		
Asia	277 (51.4%)	35/277 (12.6%)	1.093 (0.501-2.385)	0.824		
Multiple continents	10 (1.9%)	0/10 (0.0%)	0	0.999		
Reason for travel						
Vacation	468 (86.8%)	53/468 (11.3%)	1			
Business/work or internship	40 (7.4%)	5/40 (12.5%)	1.119 (0.420-2.980)	0.823		
Other	31 (5.8%)	7/31 (22.6%)	2.284 (0.939-5.557)	0.069		
Travel duration (days)	20 [15-26]	21 [15-28]	1.011 (0.996-1.026)	0.166	0.989 (0.964-1.014)	0.381

Supplementary Table 2 *Continued*

	All travellers at risk (n=539)*	Travellers who developed PI-IBS (n=65, n/N%)**	OR (95% CI)	P-value	Adjusted OR (95% CI)	Adjusted p-value
General health prior to travel						
Excellent	155 (28.9%)	11/155 (7.1%)	1			
Good	331 (61.8%)	45/331 (13.6%)	2.060 (1.034-4.102)	0.04	0.884 (0.326-2.394)	0.808
Reasonable, mediocre or bad	50 (9.3%)	8/50 (16.0%)	2.494 (0.942-6.600)	0.066	0.772 (0.166-3.594)	0.741
Depressive feelings up to three months prior to travel						
No	470 (87.2%)	55/470 (11.7%)	1			
Yes	69 (12.8%)	10/69 (14.5%)	1.304 (0.630-2.700)	0.475		
Antibiotics up to three months before travel						
No	478 (89.0%)	58/478 (12.1%)	1			
Yes	59 (11.0%)	7/59 (11.9%)	0.975 (0.423-2.248)	0.952		
Heart burn up to three months prior to travel						
No	427 (79.2%)	46/427 (10.8%)	1			
Yes	112 (20.8%)	19/112 (17.0%)	1.692 (0.947-3.024)	0.076	1.29 (0.577-2.886)	0.535
Feeling tired up to three months prior to travel						
No	314 (58.3%)	23/314 (7.3%)	1			

	All travellers at risk (n=539)*	**Travellers who developed PI-IBS** (n=65, n/N%)**	**OR (95% CI)**	**P-value**	**Adjusted OR (95% CI)**	**Adjusted p-value**
Yes	225 (41.7%)	42/225 (18.7%)	2.904 (1.690-4.988)	<.001	1.785 (0.872-3.653)	0.113
Stomach ache up to three months prior to travel						
No	436 (80.9%)	41/436 (9.4%)	1			
Yes	103 (19.1%)	24/103 (23.3%)	2.927 (1.674-5.117)	<.001		
Stomach cramps up to three months prior to travel						
No	447 (82.9%)	42/447 (9.4%)	1			
Yes	92 (17.1%)	23/92 (25.0%)	3.214 (1.820-5.677)	<.001	1.276 (0.568-2.864)	0.555
Vomiting in the past three months prior to travel						
No	495 (91.8%)	55/495 (11.1%)	1			
Yes	44 (8.2%)	10/44 (22.7%)	2.353 (1.102-5.025)	0.027	2.456 (0.864-6.983)	0.092
Nausea in the past three months prior to travel						
No	457 (84.8%)	46/457 (10.1%)	1			

Supplementary Table 2 *Continued*

	All travellers at risk (n=539)*	**Travellers who developed PI-IBS** (n=65, n/N%)**	**OR (95% CI)**	**P-value**	**Adjusted OR (95% CI)**	**Adjusted p-value**
Yes	82 (15.2%)	19/82 (23.2%)	2.695 (1.484-4.894)	0.001	1.215 (0.51-2.895)	0.66
Chronic illness						
No	419 (78.3%)	46/419 (11.0%)	1			
Yes	116 (21.7%)	19/116 (16.4%)	1.588 (0.890-2.835)	0.118	2.348 (1.075-5.131)	0.032
Diet						
No diet	486 (90.2%)	51/486 (10.5%)	1			
Vegetarian or Vegan	31 (5.8%)	8/31 (25.8%)	2.967 (1.261-6.977)	0.013	3.485 (1.132-10.731)	0.03
Other diet (Jewish, Islamic or other)	22 (4.1%)	6/22 (27.3%)	3.199 (1.198-8.540)	0.02	5.692 (1.556-20.824)	0.009
Probiotics use up till 12 months prior to travel (capsules/tablets/in dairy)						
No	315 (59.0%)	35/315 (11.1%)	1			
Seldom	113 (21.2%)	16/113 (14.2%)	1320 (0.699-2.490)	0.392		
Occasionally	58 (10.9%)	5/58 (8.6%)	0.755 (0.283-2.015)	0.574		
Often or daily	48 (9.0%)	8/48 (16.7%)	1.600 (0.693-3.693)	0.271		
Anxiety up to three months prior to travel						
No	505 (93.7%)	57/505 (11.3%)	1			

	All travellers at risk (n=539)*	**Travellers who developed PI-IBS** (n=65, n/N%)**	**OR (95% CI)**	**P-value**	**Adjusted OR (95% CI)**	**Adjusted p-value**
Yes	34 (6.3%)	8/34 (23.5%)	2.418 (1.045-5.596)	0.039	1.46 (0.44-4.847)	0.537
Main accommodation during travel						
Multiple accommodations	164 (30.4%)	20/164 (12.2%)	1			
Luxury stay (all-inclusive/4-5 stars)	103 (19.1%)	12/103 (11.7%)	0.949 (0.443-2.035)	0.894		
Hotel or apartment (0-3 stars)	154 (28.6%)	19/154 (12.3%)	1.013 (0.518-1.981)	0.969		
Low budget (guesthouse/hostel)	67 (12.4%)	10/67 (14.9%)	1.263 (0.557-2.864)	0.576		
Family/locals	25 (4.6%)	2/25 (8.0%)	0.626 (0.137-2.859)	0.546		
Tent. ship or other	26 (4.8%)	2/26 (7.7%)	0.600 (0.132-2.734)	0.509		
Antibiotics use during and/or directly after travel						
No	486 (90.8%)	54/486 (11.1%)	1			
Yes	49 (9.2%)	10/49 (20.4%)	2.051 (0.969-4.343)	0.06	2.957 (1.141-7.662)	0.026
General health during travel						
Excellent	140 (26.2%)	6/140 (4.3%)	1			
Good	343 (64.2%)	49/343 (14.3%)	3.722 (1.556-8.902)	0.003	5.09 (1.587-16.326)	0.006

Supplementary Table 2 *Continued*

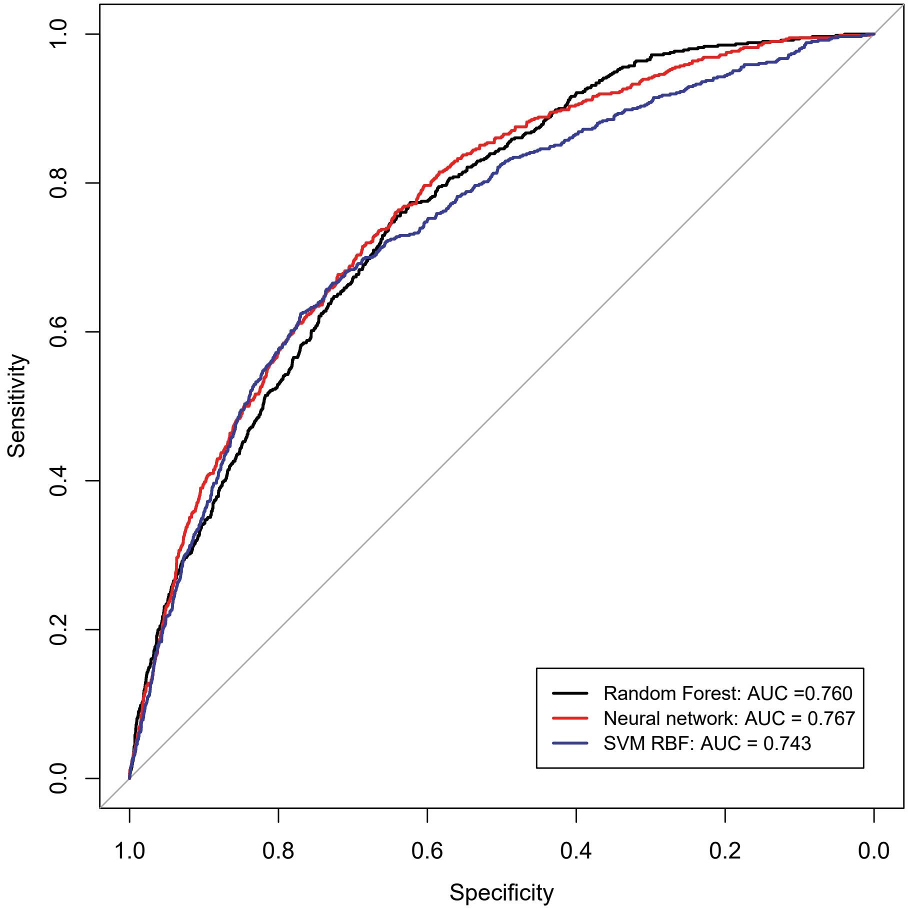
	All travellers at risk (n=539)*	**Travellers who developed PI-IBS** (n=65, n/N%)**	**OR (95% CI)**	**P-value**	**Adjusted OR (95% CI)**	**Adjusted p-value**
Reasonable, mediocre, or bad	51 (9.6%)	10/51 (19.6%)	5.447 (1.867-15.894)	0.002	5.474 (1.232-24.329)	0.026
Anxiety during travel						
No	513 (95.2%)	59/513 (11.5%)	1			
Yes	26 (4.8%)	6/26 (23.1%)	2.308 (0.891-5.980)	0.085	1.314 (0.349-4.95)	0.687
Diarrhoea inhibitors during travel						
No	336 (62.3%)	38/336 (11.3%)	1			
Yes	203 (37.7%)	27/203 (13.3%)	1.203 (0.710-2.038)	0.492		
Antacids use during travel						
No	510 (94.6%)	55/510 (10.8%)	1			
Yes	29 (5.4%)	10/29 (34.5%)	4.354 (1.927-9.839)	<.001	1.687 (0.503-5.657)	0.397
Feeling tired during travel						
No	308 (57.1%)	27/308 (8.8%)	1			
Yes	231 (42.9%)	38/231 (16.5%)	2.049 (1.211-3.468)	0.008	0.662 (0.313-1.4)	0.28
Heart burn during travel						
No	458 (85.0%)	45/458 (9.8%)	1			
Yes	81 (15.0%)	20/81 (24.7%)	3.009 (1.666-5.436)	<.001	1.349 (0.564-3.23)	0.501

	All travellers at risk (n=539)*	Travellers who developed PI-IBS (n=65, n/N%)**	OR (95% CI)	P-value	Adjusted OR (95% CI)	Adjusted p-value
Vomiting during travel						
No	463 (85.9%)	49/463 (10.6%)	1			
Yes	76 (14.1%)	16/76 (21.1%)	2.253 (1.205-4.213)	0.011		
Stomach ache during travel						
No	314 (58.3%)	20/314 (6.4%)	1			
Yes	225 (41.7%)	45/225 (20.0%)	3.675 (2.102-6.424)	<.001		
Weight loss during travel						
No	410 (76.1%)	37/410 (9.0%)	1			
Yes	129 (23.9%)	28/129 (21.7%)	2.795 (1.632-4.786)	<.001	2.241 (1.068-4.702)	0.033
Nausea during travel						
No	350 (64.9%)	21/350 (6.0%)	1			
Yes	189 (35.1%)	44/189 (23.3%)	4.754 (2.728-8.284)	<.001	2.249 (1.078-4.693)	0.031
Stomach cramp during travel						
No	209 (38.8%)	7/209 (3.3%)	1			
Yes	330 (61.2%)	58/330 (17.6%)	6.153 (2.751-13.764)	<.001	5.915 (2.17-16.121)	<0.001
Obstipation during travel						

Supplementary Table 2 *Continued*

	All travellers at risk (n=539)*	**Travellers who developed PI-IBS** (n=65, n/N%)**	**OR (95% CI)**	**P-value**	**Adjusted OR (95% CI)**	**Adjusted p-value**
No	445 (82.6%)	44/445 (9.9%)	1			
Yes	94 (17.4%)	21/94 (22.3%)	2.622 (1.473-4.666)	0.001	1.503 (0.687-3.29)	0.308
Fever during travel						
No	487 (90.4%)	55/487 (11.3%)	1			
Yes	52 (9.6%)	10/52 (19.2%)	1.870 (0.888-3.938)	0.099		
Depressive feelings during travel						
No	512 (95.0%)	59/512 (11.5%)	1			
Yes	27 (5.0%)	6/27 (22.2%)	2.194 (0.851-5.655)	0.104		

* And ** numbers do not add up to 539 or 65 due to missing numbers. Missing values were assumed to be missing at random. The numerator represents the number of travellers who acquired PI-IBS after traveller’s diarrhoea (TD) and the denominator represent the number of travellers at risk. IQR, interquartile range.



Supplementary Figure 1. Overall receiver operating characteristic (ROC) curves from random forest, neural network, and support vector machine models. AUC = area under receiver operating characteristic

CHAPTER 4

Gut microbiota dynamics during intercontinental travel and its sequelae for post-infection irritable bowel syndrome

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Manuscript in preparation

CHAPTER 5

Antimicrobial resistance genes acquisition among Dutch intercontinental travellers: a prospective multicentre study

Based on:
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Abstract

Knowledge about the facilitators of antimicrobial resistance (AMR) gene acquisition during travel and the role of the human gut microbiota as a reservoir remains limited. This study investigated AMR gene acquisition among travellers, focusing on pre- and post-travel prevalence and associated risk factors. Using data from 637 intercontinental travellers in the prospective COMBAT study, we screened faecal samples collected before and after travel for AMR genes using PCR-based targeted metagenomics. The prevalence of genes encoding extended-spectrum beta-lactamase, quinolone resistance, colistin resistance, and aminoglycoside resistance significantly increased post-travel ($p<0.05$). Statistical analyses, including Pearson's chi-square test, Multiple Correspondence Analysis (MCA), and logistic regression models, were employed to explore associations and identify risk factors for $bla_{CTX\text{-}M\text{-}(1,\ 2\ and\ 9\ groups)}$, *mcr (1, 3, 4, and 5), qnrS*, and *qnrB* acquisition. Risk factors for AMR gene acquisition varied by gene but commonly included region visited, antibiotic use, chronic disease, and consumption of shellfish. These findings reinforce that AMR gene acquisition among travellers is both frequent and significant, with implications for global AMR surveillance and travel medicine.

Introduction

The continuous rise in antimicrobial resistance (AMR) is a major public health threat globally. The prevalence of AMR, however, varies between regions[1], with low- and middle-income countries often having higher rates of AMR due to excessive antibiotic consumption and socioeconomic factors[2,3]. People from regions with low AMR prevalence that travel to high-prevalence areas frequently acquire AMR bacteria in their gut, importing them to their home country[4,5].

Several reports and systematic reviews have been published on travel, risk factors and acquisition of AMR[6-9]. These studies particularly focused on clinically relevant indicator bacteria in the human gut, often opportunistic pathogens, with genes encoding for extended-spectrum beta-lactamases (ESBL). However, a largely unexplored area is the reservoir of AMR genes harboured by non-pathogenic commensal microorganisms present in the human gut microbiota[10]. Bacteria in the densely populated gut are able to exchange genetic material through horizontal gene transfer (HGT), which can lead to the spread of AMR genes between commensals and opportunistic pathogens[11]. In a traveller setting, the gastrointestinal tract is constantly exposed to various types of bacteria in foreign environments, potentially containing antibiotic resistance genes. The bacteria encoding these genes might persist and colonize themselves or through HGT transmit their resistance genes to indigenous microorganisms and thereby add to the pool of AMR genes residing in the gut microbiome, collectively termed “the gut resistome”[10,12].

It is therefore important not only to assess AMR in individual indicator organisms, but also to examine AMR within the commensal gut ecosystem. Molecular methods such as metagenomics and quantitative PCR (qPCR) bypass culture-based limitations by enabling detection of AMR genes in both culturable and unculturable bacteria. Metagenomics can reveal the broader ARG repertoire of a sample without predefined targets, yet its value is limited by workflow variability, semiquantitative outputs and reduced sensitivity for low abundance genes[13,14]. qPCR, in contrast, provides a sensitive targeted approach to monitor ARG acquisition.

The aim of this study was to prospectively investigate travel related risk factors for the acquisition of ARGs in the gut microbiota of 637 healthy Dutch travellers using a targeted analytical approach combined with an extensive set of metadata.

Material and Methods

Study population and design

The present study was conducted within the context of the COMBAT-study, a multicentre prospective cohort study involving 2001 Dutch travellers recruited at several outpatient clinics from November 2012 until November 2013[6,15].

Participants were instructed to self-collect stools through faecal swabs (Fecal Swab®; Copan, Brescia, Italy) and filled in questionnaires before and immediately after travel. The questionnaires contained information on potential predictors for AMR acquisition. Faecal material was stored at -80 °C in Cary Blair medium.

Only participants who provided a written informed consent were enrolled. Ethical approval was obtained by the Medical Ethical Committee of Maastricht University Medical Centre (study number: METC 12-4-093). A full description of the study design and methods have been described elsewhere[6,15]. This study builds on earlier work that identified regional differences in AMR gene acquisition among 190 participants in the COMBAT study who travelled to South Eastern Asia, South Asia, North Africa or Eastern Africa[16]. For the current analysis, we selected 637 travellers who visited one of these four sub regions, excluding individuals who travelled to more than one region.

DNA extraction, detection, and quantification of AMR genes

DNA was extracted from biobanked faecal samples by a combination of repeated bead-beating and column-based purification according to protocol Q of the International Human Microbiome Standards consortium[17,18].

To determine the bacterial load, we enumerated the total number of 16S rRNA gene copies using a MYiQ™ Single-Color Real-Time PCR detection system (BioRad, Hercules, CA, USA)[19]. Identification and quantification of the resistance genes was done using (in-house designed) PCR primers and probes following previously described and validated protocols (Supplementary Table 1)[20-23].

Acquisition of AMR genes was defined as absence of AMR genes before travel and presence of AMR genes after travel in faecal samples. Presence was defined as any reaction yielding a quantifiable Ct value below the Ct observed in the negative isolation control; samples with no detectable amplification or with Ct values ≥ that of the negative control were classified as absent.

Statistical analysis

To evaluate the acquisition of AMR genes, we performed a McNemar's test on paired data to compare AMR prevalence before (T0) and after (T1) travel.

The association between acquisitions of multiple AMR genes was determined by Pearson's chi-squared tests and the relationships were further explored with multiple correspondence analysis (MCA). MCA is an exploratory principal component technique designed to explore the relationship among multiple variables using a graphical representation[24-26]. In short, values of a table of relative frequency were distributed in an n-dimensional space and the degree of similarity between variables was examined through observing the distances between variables in the dimensions.

Bivariable and multivariable logistic regression models were used to identify predictors associated with AMR gene acquisition during travel, similar to the purposeful selection process proposed by Hosmer and Lemeshow[27,28]. According to previous studies[6,21], travel destination is a strong confounder and therefore a bivariable logistic regression analyses, including travel destination plus one other predictor, was performed to select variables (p-value <0.25) for the multivariable analysis. Variables were tested for multicollinearity and interaction with sub regions and those that showed interaction were removed before the multivariable analysis.

In the multivariable logistic regression, we performed stepwise removal of variables that were not statistically significant (Wald test $p > 0.05$), beginning with the variable with the highest p-value. After each removal, the model was refitted and the parameter estimates of the remaining variables were compared with those from the full model. Variables were retained if their removal changed any parameter estimate by ≥20%, indicating that they acted as confounders and provided essential adjustment. Variables whose removal resulted in <20% change and were statistically insignificant were eliminated from the model[27]. After this iterative process, the final models only included significant variables ($p<0.05$) and confounders. Results are presented as odds ratios (ORs) and accompanying 95% confidence intervals (CIs).

All analyses, except for MCA, were performed using IBM SPSS Statistics (version 27) and the results were interpreted as statistically significant when $p<0.05$. MCA was performed using R (version 4.0.3) and R-packages FactoMineR[29] and factoextra[30].

Results

637 travellers were included in the study with median age 51.5 years (IQR 34.9-60.5) of which 53.6% (341/637) were female (Table 1). The median travel duration was 20 days (IQR 15-23) and the main purpose for travel was tourism (87.3%). South East Asia was the most visited sub region with 346 travellers (54.3%), followed by South Asia with 127 travellers (19.9%), East Africa (119, 18.7%) and North Africa (45, 7.1%). Among the 637 participants, 2 individuals (0.3%) used antibiotics prior to travel and 8 (1.3%) had a chronic bowel disease.

Table 1. Baseline characteristics of travelers.

Characteristics	Travellers (N = 637)*
Sex	
Male	295 (46.4%)
Female	341 (53.6%)
Age (median, in years [IQR])	51.5 [34.9:60.5]
Education level	
No education, elementary school or pre-vocational secondary education	71 (11.2%)
Vocational secondary education or pre-university education	153 (24.2%)
Higher professional education	216 (34.0%)
Academic (university) education	195 (30.7%)
Chronic disease	
No	500 (79.5%)
Yes	129 (20.5%)
Chronic bowel disease	
No	629 (98.7%)
Yes	8 (1.3%)
Antibiotics use within three months prior to travel	
No	635 (99.7%)
Yes	2 (0.3%)
Subregion visited during travel	
East Africa	119 (18.7%)
North Africa	45 (7.1%)
South Asia	127 (19.9%)
South East Asia	346 (54.3%)
Purpose of travel	
Holiday	556 (87.3%)
Work/internship	33 (5.2%)
Visit to family or friends	24 (3.8%)
Other	24 (3.8%)
Travel duration (median, in days [IQR])	20 [15:23]

IQR: interquartile range
* Some numbers do not add up to 637 due to missing data.

Prevalence of AMR genes in stool samples

Before travel, antibiotic resistance genes were already present in many participants (Figure 1 and Supplementary Table 2). The prevalence of *cfxA*, *tetM*, *tetQ*, *ermB*, *ermF* and *mefAE* was found to be high in the pre-travel faecal samples, ranging from 80.3-99.7%, and was not altered post-travel. The prevalence of the aminoglycoside resistance gene aac(6')-aph(2'') significantly increased from 339 (53.2%) before travel to 365 (57.3%) after travel ($p<0.05$). Carbapenemase genes *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{NDM-1}}$ were only detected sporadically in both pre- as well as post-travel samples.

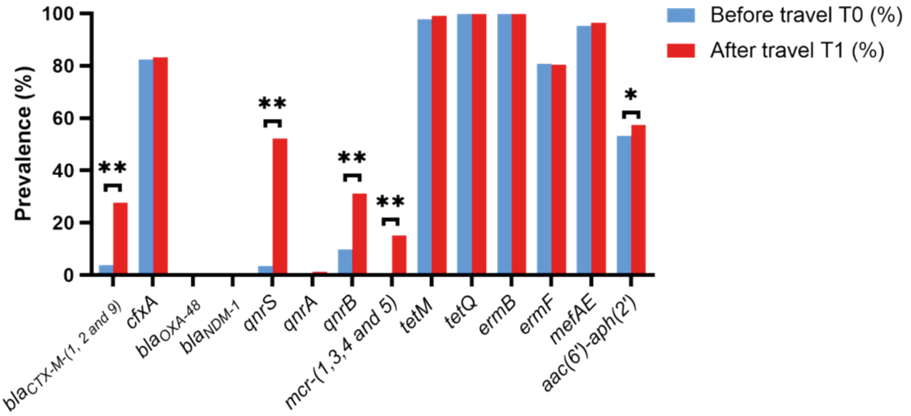


Figure 1. Prevalence (%) of antibiotic resistance genes in stool samples from Dutch travellers (N=637) before and after travel. Not all numbers add up to 637 stool samples due to missing data (See Supplementary table 2 for full data). *Indicates $p<0.05$ and **indicates $p<0.001$ as calculated using McNemar's test for paired samples.

The extended spectrum beta-lactamase (ESBL) encoding genes *bla*$_{\text{CTX-M-(1, 2 and 9 groups)}}$ (3.8%), the quinolone resistance genes *qnrA* (0%), *qnrB* (9.9%) and *qnrS* (3.5%) and the colistin resistance genes *mcr-(1, 3, 4 and 5)* (0.3%) all had low prevalence in the pre-travel faecal samples. A statistically significantly higher proportion of post-travel stool samples contained *mcr-1* (13.0%), *mcr-2* (2.5%), *mcr-5* (3.1%), *bla*$_{\text{CTX-M-1}}$ (20.1%), *bla*$_{\text{CTX-M-2}}$ (2.0%) and *bla*$_{\text{CTX-M-9}}$ (9.1%) (all $p<0.005$, Supplemental table 2). An exception was *qnrA* with an 1.2% presence in post-travel samples compared to 0% in pre-travel samples which was increased albeit statistically insignificant ($p = 0.125$).

Association between AMR genes

Because the prevalence of *bla*$_{\text{CTX-M-(1, 2 and 9 groups)}}$, *qnrB*, *qnrS* and *mcr- (1, 3, 4 and 5)* were statistically significantly higher in the post-travel stool samples compared to pre-travel($p<0.001$); we limited our subsequent analysis to these genes.

First, we examined the association between the acquisitions of these individual AMR genes (or groups of genes belonging to the same AMR type e.g., *bla*$_{\text{CTX-M-(1, 2 and 9 groups)}}$). Travelers who carried any of these genes before travel were excluded. Subsequently, the gene acquisition profile of 540 travellers was compared using Pearson chi-square testing. The results showed that all genes included in the analysis were associated with each other (all p<0.05, Supplementary Table 3). To further explore these associations, a Multiple Correspondence Analysis (MCA) was performed on the AMR genes acquisition frequencies and displayed in variable correlation plots (Figure 2).

The first three MCA dimensions in the analysis accounted for 83.8% of the variance in the data (Figure 2A). Dimension 1 constituted the largest part of the variance (39.9%) followed by dimension 2 (23.2%) and dimension 3 (20.7%). The closer the AMR gene acquisition profiles are located on the plane, the more correlated they are. Squared cosines (cos2) were computed to indicate the variables that were best represented in the dimensions (Figure 2B-2D). Subsequently, the contribution of variables to the inertia of the axes was determined (Figure 2E-2H). Dimension 1 separated pre-travel samples without AMR genes (denoted 0) from post-travel samples with acquired AMR genes (denoted 1). In addition, there was a separation between post-travel AMR genes along the second and third dimensions (Figure 2B and 2C respectively) which indicated that there were relative differences in associations between *bla*$_{\text{CTX-M-(1, 2 and 9 groups)}}$, *mcr-(1,3,4 and 5)*, *qnrS*- and *qnrB* acquisition.

The results further showed that there was less association between *mcr-(1,3,4 and 5)* and *bla*$_{\text{CTX-M-(1, 2 and 9 groups)}}$ and between *mcr-(1,3,4 and 5)* and *qnrB* compared to other combinations of AMR genes in the analysis. In addition, the post-travel AMR genes were not consistently clustered together across the first three dimensions (Figure 2A-2C). These results demonstrated that associations between AMR genes, or groups of AMR genes belonging to the same type, were not uni-dimensional, and they were not necessarily simultaneously acquired during travel. Subsequent predictor identification through logistic regression analysis was performed separately for each AMR gene or group of genes.

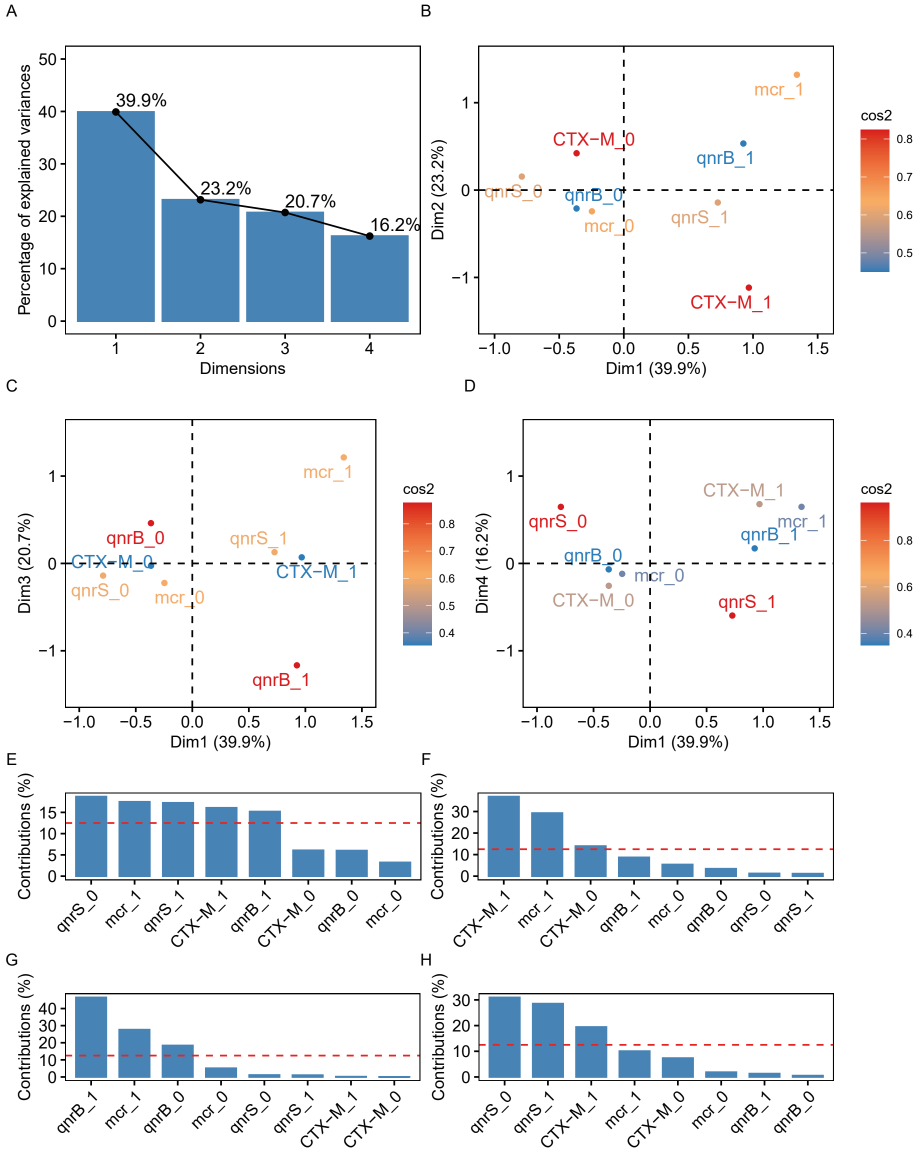


Figure 2. Relationship among the AMR genes displayed in MCA graphs. **A)** Screeplot showing the percentages of explained variance per dimension. **B-D**) Correlation plots of variables (AMR gene or group of genes belonging to the same AMR type) on dimension 1 and dimension 2, 3 or 4. The distance is inversely proportional to the correlation between variables. Percentages for each axis indicate the proportion of explained variance in each dimension. Squared cosines (cos2) were computed to indicate the variables that were best represented in the dimensions with. Quality of the variables in cosine^2 normalized between 0 and 1 (near 1 indicates a strong representation while near 0 indicates a weak representation of the variable in the dimensional space). **E-H)** Contribution of the variables on the first, second, third or fourth dimension

expressed in %. The dotted red lines indicate the expected average value if all variables contributed uniformly. CTX-M = $bla_{\text{CTX-M- (1, 2 and 9)}}$; mcr = mcr-(1, 3, 4 and 5); qnrS = qnrS; qnrB = qnrB; Dim = dimension; suffix 0 = pre-travel absence of AMR gene; suffix 1 = acquisition of AMR gene during travel.

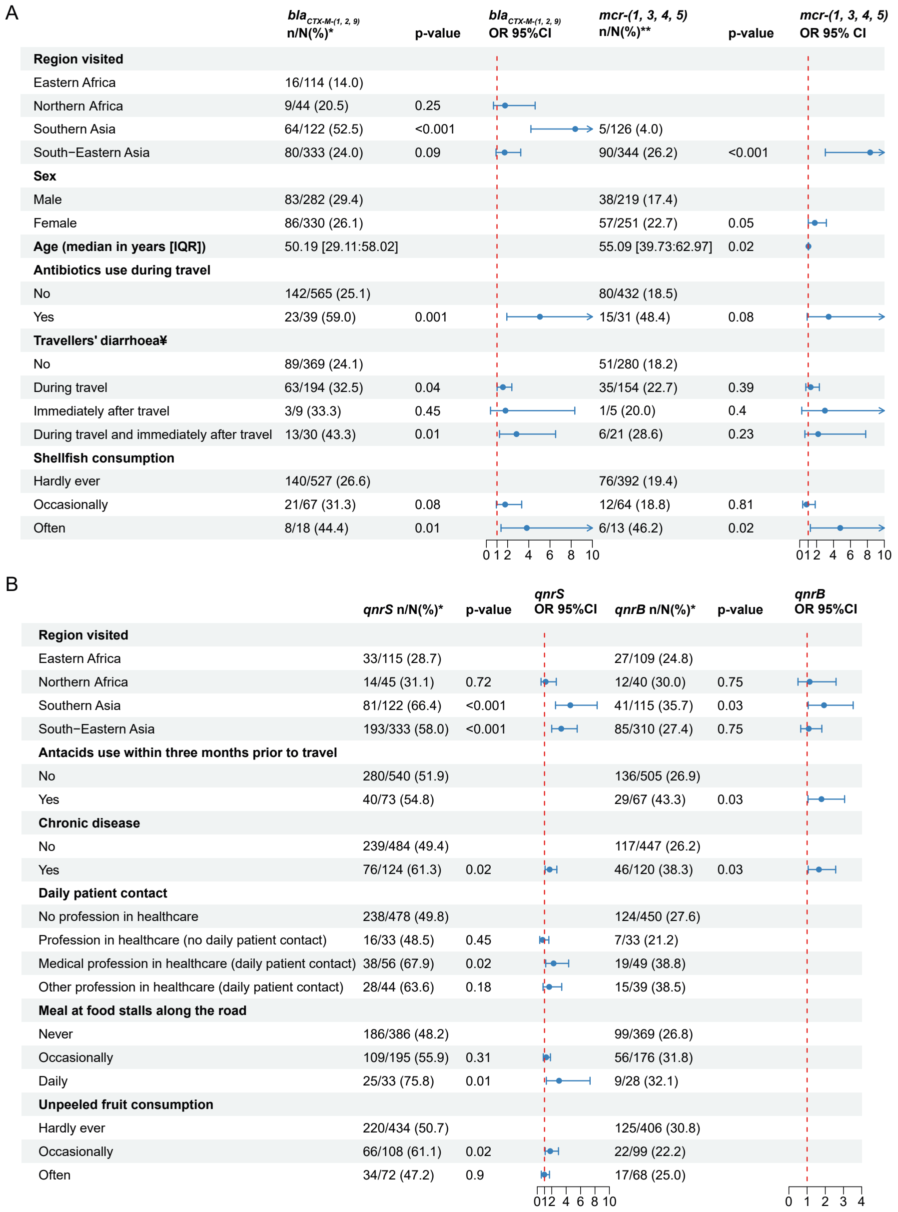
Risk factors for AMR gene acquisition

In the multivariable logistic regression (Figure 3A and Supplementary Table 4), independent risk factors associated with $bla_{\text{CTX-M-(1, 2 and 9 groups)}}$ acquisition were visiting Southern Asia (OR 8.38, 95% CI 4.20-16.69; reference was Eastern Africa), antibiotic use during travel (OR 5.05, 95% CI 1.93-13.15), diarrhoea during travel (OR 1.56, 95% CI 1.01-2.40), diarrhoea that persisted upon return (OR 2.84, 95% CI 1.24-6.53) and frequent shellfish consumption during travel (OR 3.81, 95% CI 1.38-10.52).

The acquisition of the *qnrS* gene (Figure 3B and Supplementary Table 6) was positively associated with travel to Southern Asia (OR 4.58, 95% CI 2.53-8.30); South-Eastern Asia (OR 3.32, 95% CI 2.00-5.53 with Eastern Africa as reference), having a chronic disease (OR 1.71, 95% CI 1.08-2.71), having a medical profession in healthcare with daily patient contact (OR 2.26, 95% CI 1.17-4.35), eating meal at food stalls (OR 3.02, 95% CI 1.25-7.32) and occasional unpeeled fruit consumption (OR 1.79, 95% CI 1.09-2.94).

Factors associated with a higher risk of *qnrB* gene acquisition (Supplementary Table 7) were visiting Southern Asia (OR 1.93, 95% CI 1.06-3.53 with Eastern Africa as reference), antacid use prior to travel (OR 1.79, 95% CI 1.05-3.06) and having a chronic disease (OR 1.65, 95% CI 1.06-2.57).

As *mcr*-genes were only acquired among travellers to Asia, we limited the risk factor analyses for *mcr*-gene acquisition to the 473 travellers visiting this continent (Supplementary Table 5). Among this subgroup, risk factors for *mcr-(1,3,4 and 5)* gene acquisition was visiting South-Eastern Asia (OR 7.35, 95% CI 2.83-19.08 with Southern Asia as reference), being female (OR 1.73, 95% CI 1.04-2.90), age (OR 1.03 ,95% CI 1.01-1.04), antibiotic use during travel (OR 5.16, 95% CI 2.12-12.56) and frequent shellfish consumption during travel (OR 3.80, 95% CI 1.05-13.70).



A

	bla$_{CTX\text{-}M\text{-}(1,\ 2,\ 9)}$ n/N(%)*	p-value	*bla*$_{CTX\text{-}M\text{-}(1,\ 2,\ 9)}$ OR 95%CI	*mcr-(1, 3, 4, 5)* n/N(%)**	p-value	*mcr-(1, 3, 4, 5)* OR 95% CI
Region visited						
Eastern Africa	16/114 (14.0)					
Northern Africa	9/44 (20.5)	0.25				
Southern Asia	64/122 (52.5)	<0.001		5/126 (4.0)		
South−Eastern Asia	80/333 (24.0)	0.09		90/344 (26.2)	<0.001	
Sex						
Male	83/282 (29.4)			38/219 (17.4)		
Female	86/330 (26.1)			57/251 (22.7)	0.05	
Age (median in years [IQR])	50.19 [29.11:58.02]			55.09 [39.73:62.97]	0.02	
Antibiotics use during travel						
No	142/565 (25.1)			80/432 (18.5)		
Yes	23/39 (59.0)	0.001		15/31 (48.4)	0.08	
Travellers' diarrhoea¥						
No	89/369 (24.1)			51/280 (18.2)		
During travel	63/194 (32.5)	0.04		35/154 (22.7)	0.39	
Immediately after travel	3/9 (33.3)	0.45		1/5 (20.0)	0.4	
During travel and immediately after travel	13/30 (43.3)	0.01		6/21 (28.6)	0.23	
Shellfish consumption						
Hardly ever	140/527 (26.6)			76/392 (19.4)		
Occasionally	21/67 (31.3)	0.08		12/64 (18.8)	0.81	
Often	8/18 (44.4)	0.01		6/13 (46.2)	0.02	

B

	qnrS n/N(%)*	p-value	*qnrS* OR 95%CI	*qnrB* n/N(%)*	p-value	*qnrB* OR 95%CI
Region visited						
Eastern Africa	33/115 (28.7)			27/109 (24.8)		
Northern Africa	14/45 (31.1)	0.72		12/40 (30.0)	0.75	
Southern Asia	81/122 (66.4)	<0.001		41/115 (35.7)	0.03	
South−Eastern Asia	193/333 (58.0)	<0.001		85/310 (27.4)	0.75	
Antacids use within three months prior to travel						
No	280/540 (51.9)			136/505 (26.9)		
Yes	40/73 (54.8)			29/67 (43.3)	0.03	
Chronic disease						
No	239/484 (49.4)			117/447 (26.2)		
Yes	76/124 (61.3)	0.02		46/120 (38.3)	0.03	
Daily patient contact						
No profession in healthcare	238/478 (49.8)			124/450 (27.6)		
Profession in healthcare (no daily patient contact)	16/33 (48.5)	0.45		7/33 (21.2)		
Medical profession in healthcare (daily patient contact)	38/56 (67.9)	0.02		19/49 (38.8)		
Other profession in healthcare (daily patient contact)	28/44 (63.6)	0.18		15/39 (38.5)		
Meal at food stalls along the road						
Never	186/386 (48.2)			99/369 (26.8)		
Occasionally	109/195 (55.9)	0.31		56/176 (31.8)		
Daily	25/33 (75.8)	0.01		9/28 (32.1)		
Unpeeled fruit consumption						
Hardly ever	220/434 (50.7)			125/406 (30.8)		
Occasionally	66/108 (61.1)	0.02		22/99 (22.2)		
Often	34/72 (47.2)	0.9		17/68 (25.0)		

Figure 3. Predictors for AMR genes acquisition among travellers in multivariable logistic regression models (n = 637). A) bla$_{\text{CTX-M- (1, 2 and 9)}}$ or mcr-(1,3,4 and 5) acquisition predictors B) qnrS or qnrB acquisition predictors.* = Included only travellers at risk of acquiring AMR genes in the analysis (bla$_{\text{CTX-M- (1, 2 and 9)}}$: n = 613; qnrS: n = 615; qnrB: n = 574); ** = Limited to the at risk travellers visiting Asia (mcr-(1,3,4 and 5): n = 470); 95% CI = 95% confidence interval; ¥ = Travellers' diarrhoea was characterized as three or more loose stools within a 24-hour timeframe, with or without accompanying symptoms; Hardly ever = never or less than once a week; Occasionally = approximately once a week; Often = multiple times a week or daily.

Discussion

Results from this cohort study with intercontinental Dutch travellers provided insights in the prevalence and risks of AMR gene acquisition in the gut microbiota. Significant increases in prevalence were observed for ESBL encoding genes, quinolone resistance-, colistin resistance- and aminoglycoside resistance encoding genes. This is consistent with a previous study with 122 Dutch travellers showing similar results where the prevalence of $bla_{\text{CTX-M}}$, *qnrB* and *qnrS* genes rose post-travel[21]. Interestingly, the post-travel prevalence of *mcr-1* (13.0%, 83 of 637, Supplemental table 2) was higher than previously reported by Von Wintersdorff and colleagues[23]. It could be explained by the larger sample size of 637 travellers included in our study compared to the previous study where 6 out of 122 (4.9%) had acquired *mcr-1*. Furthermore, a large part of the participants in our study acquired *mcr-1* during their visit to South-Eastern Asia. This is consistent with a study performed by Bich et al. who detected *mcr-1* prevalence rates as high as 88% and 93% in human and animal stools in a rural community cohort in Vietnam[20].

Next, our study showed that there were significant associations between $bla_{\text{CTX-M-(1, 2 and 9 groups)}}$, *qnrB*, *qnrS* and *mcr-(1,3, 4 and 5)* acquisition. $bla_{\text{CTX-M-(1, 2 and 9 groups)}}$, *qnrB* and *qnrS* were most often acquired by travellers visiting Southern Asia followed by South-Eastern Asia. Additionally, acquisition of *mcr-(1,3, 4 and 5)* genes was highest for visitors to South-Eastern Asia. These results suggest that $bla_{\text{CTX-M}}$, *qnrB*, *qnrS* and *mcr* genes potentially co-occur. This is further strengthened by the evidence that quinolone resistance is often part of mobile genetic elements carried by plasmids in combination with other antibiotic resistance genes, particularly those that code for ESBLs and colistin resistance[31-33].

Previous culture-based studies on *E. coli* isolates reported comparable risk factors for $bla_{\text{CTX-M}}$ acquisition during travel as were found in our current study. Arcilla *et al.* and other studies summarized by meta-analyses and systematic reviews presented travelling to Southern Asia, antibiotic use during travel and traveller's diarrhoea as major risk factors for the acquisition of $bla_{\text{CTX-M}}$[6,8,9]. In addition, we found that frequent (several times a week, or daily) consumption of shellfish contributes to a higher risk of acquiring $bla_{\text{CTX-M-(1, 2 and 9 groups)}}$ during travel. Studies in Asia have reported antibiotics resistant bacteria in shellfish that harboured genes conferring resistance to antimicrobial agents such as cephalosporins[34-36]. $Bla_{\text{CTX-M-(1, 2 and 9 groups)}}$ encodes for ESBLs that hydrolyse cephalosporins. It is likely that travellers who consumed shellfish containing such ESBL encoding bacteria acquired *bla*$_{\text{CTX-M}}$ genes through Horizontal Gene Transfer (HGT).

The consumption of shellfish is similarly a risk factor for *mcr* gene acquisition during travel. A recent study found plasmid-mediated *mcr-1* gene in *Vibrio parahaemolyticus*, a potential human pathogen isolated from seafood samples in China[37]. This study further highlights the possibility of HGT of AMR genes between different bacteria species, which could explain the occurrence of *mcr-1* genes in the human gut resistome in our participants through consumption of shellfish. Cabello et al. discussed in their paper the overuse of antimicrobials in the aquaculture and combined with the increased industrialization this may lead to a new source for AMR transmission between aquatic animals and humans[38]. Our study suggests that this transmission already occurs in a global setting supported through travel. However, a larger study sample size is needed for more robust results given the wide confidence intervals in our study.

These findings, which indicate that consuming shellfish during travel may be a risk factor for acquiring *bla*$_{\text{CTX-M-(1, 2 and 9 groups)}}$ and *mcr (1, 3, 4 and 5),* may stem from the use of a qPCR-based approach applied directly to whole-sample DNA. This technique can reveal a wider spectrum of AMR genes than studies that rely only on cultured Enterobacteriaceae, which may miss genes present in non-culturable or low-abundant organisms[21].

The study by Von Wintersdorff et al. study reported comparable predictors associated with the acquisition of *qnr*. Visiting Southern- and South Eastern Asia were risk factors for the acquisition of *qnrS* during travel[21]. Interestingly, the added benefit of our increased cohort size revealed additional determinants. Having a chronic disease, having a medical profession in healthcare with daily patient contact, eating daily meals at food stalls and occasionally eating unpeeled fruit increased the risk of acquiring *qnrS* gene during travel. In addition, prior use of antacids before travel and having a chronic disease contributed to an increased risk of *qnrB* acquisition during travel.

Antibiotic use, drug (antacid) use, traveller's diarrhoea, and chronic (bowel) diseases have well established associations with changes in the gut microbiota, potentially inducing dysbiosis[39-41]. Subsequent potential reduction in colonization resistance may facilitate (temporal) colonization of ingested bacteria that may subsequently transfer plasmid mediated genes via HGT. Our work emphasizes the importance of pre-travel consultation to reinforce travellers to adhere to recommendations regarding hand hygiene, food and water safety precautions, and appropriate medication use during travel. The results further suggest to give attention to the interconnection of human, animal and environmental settings and their role in AMR transmission[42]. An integrated approach may reduce the global spread of AMR genes.

A potential limitation of our study is the targeted detection of a limited number of AMR genes. Furthermore, our results can neither link the AMR genes to the bacteria that harbour these genes nor inform on the functional expression of these genes. Lastly our study population was derived from participants who went to outpatient clinics before travel and may therefore be more affluent and healthier than the average population. This selection could have affected the frequency of AMR acquisition and the generalizability of our results. However, bias is unlikely if the selection does not affect the exposure and the outcome.

It would be valuable for future studies to integrate culture-independent metagenomic sequencing with targeted isolation and cultivation of intestinal microorganisms. This combined approach would allow more accurate identification of the bacterial hosts carrying antimicrobial resistance (AMR) determinants and enable screening of gut microbes for mechanisms of horizontal gene transfer (HGT). In addition to long-read metagenomics, chromosome conformation capture–based methods such as Hi-C can further help to associate mobile genetic elements with their host genomes by preserving in vivo DNA proximity[14,43]. Together, these methods would provide a more comprehensive understanding of how the gut resistome changes after travel and how AMR genes are transmitted and acquired. Currently, the dynamics of the post-travel gut resistome remain poorly characterized, highlighting the need for detailed mechanistic studies

In conclusion, our findings support the contribution of intercontinental travel to the increased acquisition of AMR when visiting low-to middle income countries. We recommend to advice travellers through tailored pre-travel counselling to minimize travel-related risk factors that predispose to AMR acquisition.

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Conflict of interest

None declared.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Supplementary materials

Supplementary Table 1. PCR primer/probe sequences to identify AMR genes in gut microbiota.

Primer/ probe	Sequence* 5' -3'	Target	Reference
16S_341_F	CCTACGGGNGGCWGCAG	16S rDNA	[19,21]
16S-805_R	GACTACHVGGGTATCTAATCC		
cfxA_F	TGACAGTGAGAGATTTGCTGC	cfxa	[21,44]
cfxA_R	GGTCAGCCGACATTTCCTCTT		
tetM_F	ACACGCCAGGACATATGGAT	tetM	[21,44]
tetM_R	GGGAATCCCCATTTTCCTAA		
tetQ_F	CAAGGTGATATCCGCTCTGA	tetQ	[21,44]
tetQ_R	GGAAAATCGTTCTTCCAGCA		
ermB_F	AAGGGCATTTAACGACGAAACTG	ermB	[21]
ermB_R	ATTTATCTGGAACATCTGTGGTATG		
aac6-aph2_F	TTGGGAAGATGAAGTTTTTAGA	aac(6')-aph(2")	[21,45]
aac6-aph2_R	CCTTTACTCCAATAATTTGGCT		
CTX-M-F	ATGTGCAGYACCAGTAARGTKATGGC		[46,47]
CTX-M-R	ATCACKCGGRTCGCCNGGRAT		
CTX-M-Probe-1	JOE-CCCGACAGCTGGGAGACGAAACGT-BHQ-1	CTX-M-1	
CTX-M-Probe-2	6-FAM-CAGGTGCTTATCGCTCTCGCTCT GTT-BHQ-1	CTX-M-2	
CTX-M-Probe-9	JOE-CTGGATGCACTGAACCTACGCTGA-BHQ-1	CTX-M-9	
blaNDM-F	ATTAGCCGCTGCATTGAT	blaNDM	[21,22,47]
blaNDM-R	CATGTCGAGATAGGAAGTG		
blaNDM-probe	6-FAM-CTG [+C]CA [+G]AC [+A]TT [+C]GG TGC-BHQ-1		
blaOXA-48-F	GTAGCAAAGGAATGGCAA	blaOXA-48	[48]
blaOXA-48-R	CCTTGCTGCTTATTGTCA		
blaOXA-48-Probe	6-FAM-TCC [+A]GA [+G]CA [+C]AA [+C]TA CG-BHQ-1		
mcr-1-F	CGGTCAGTCCGTTTGTTG	mcr-1	[23]
mcr-1-R	CTTGGTCGGTCTGTAGGG		

Primer/ probe	**Sequence* 5' -3'**	**Target**	**Reference**
mcr-1-Probe	6-FAM-TTGACCGCGACCGCCAATCTTA-BHQ-1		
mcr-3-F	GGTGAATCACTGGGAGCATTAG	mcr-3	[20]
mcr-3-R	GCTGCAAACACGCCATATC		
mcr-3-Probe	6-FAM-ACACCGTACCAGTTTGCACCGGAT-BHQ-1		
mcr-4-F	TGCTGGTTGGGTATCTAATG	mcr-4	[20]
mcr-4-R	AGTCCTAGCAAACTGTGTCGAAC		
mcr-4-Probe	JOE-TGACTTGTGTTGCACAGCGAGCAGA-BHQ-1		
mcr-5-F	GAGAATGCTGCCCTACTTGT	mcr-5	[20]
mcr-5-R	CCACAGACCCATGGAAATC		
mcr-5-Probe	6-FAM-ACGGGCTGGAAACAAGCGGTAATGA-BHQ-1		
qnrA_F	CAGTTTCGAGGATTGCAGTT	qnrA	[21,49]
qnrA_R	CCTGAACTCTATGCCAAAGC		
qnrA_probe	6FAM-AAGGGTGYCACTTCAGCTATGCC-BHQ1		
qnrB_F	CAGATTTYCGCGGCGCAAG	qnrB	[21,49]
qnrB_R	TTCCCACAGCTCRCAYTTTTC		
qnrB_probe	6FAM-CGCACCTGGTTTTGYAGYGCMTATATCAC-BHQ1	qnrS	[21,49]
qnrS_F	TCAAGTGAGTAATCGTATGTA		
qnrS_R	GTCTGACTCTTTCAGTGAT		
qnrS_probe	6FAM-CCAGCGATTTTCAAACAACTCAC-BHQ1		

*Nucleic acids between brackets and preceded by '+' are locked nucleic acids.

Supplementary Table 2. Prevalence (%) of antibiotic resistance determinants in stool samples from Dutch travellers (N=637M) before and after travel.

AMR genes[¥]	Total number of travellers*	Before travel T0 (%)	After travel T1 (%)	p-value
bla$_{\text{CTX-M-1}}$	637	14 (2.2)	128 (20.1)	<0.001
bla$_{\text{CTX-M-2}}$	637	1(0.2)	13 (2.0)	<0.01
bla$_{\text{CTX-M-9}}$	637	9 (1.4)	58 (9.1)	<0.001
bla$_{\text{CTX-M-1,2 and 9}}$	637	24 (3.8)	176 (27.6)	<0.001
cfxA	432	356 (82.4)	359 (83.1)	0.73
bla$_{\text{OXA-48}}$	637	3 (0.5)	1 (0.2)	0.63
bla$_{\text{NDM-1}}$	637	0 (0)	1 (0.2)	1.00
qnrS	637	22 (3.5)	332 (52.1)	<0.001
qnrA	330	0 (0)	4 (1.2)	0.13
qnrB	637	63 (9.9)	199 (31.2)	<0.001
mcr-1	637	2 (0.3)	83 (13.0)	<0.001
mcr-3	637	1 (0.2)	16 (2.5)	<0.001
mcr-4	637	0 (0)	1 (0.2)	1.00
mcr-5	637	0 (0)	20 (3.1)	<0.001
mcr-(1,3,4 and 5)	637	3 (0.5)	97 (15.2)	<0.001
tetM	637	623 (97.8)	631 (99.1)	0.06
tetQ	637	635 (99.7)	635 (99.7)	1.00
ermB	432	431 (99.8)	431 (99.8)	1.00
ermF	432	347 (80.3)	347 (80.3)	1.00
mefAE	430	410 (95.3)	414 (96.3)	0.54
aac(6')-aaph(2')	637	339 (53.2)	365 (57.3)	0.02

*Not all numbers add up to 637 travellers due to missing values. [¥]Travellers with multiple different mcr or bla$_{\text{CTX-M}}$ genes were only counted once when computing the total count of mcr-(1,3,4 and 5) or bla$_{\text{CTX-M-1,2 and 9}}$.

Supplementary Table 3. Associations between acquisition of AMR genes during travel by Dutch intercontinental travellers.

		***qnrS* acquisition**		***qnrB* acquisition**		***mcr-(1,3,4 and 5)* acquisition**	
		No	Yes	No	Yes	No	Yes
bla$_{\textit{CTX-M-(1,2 and 9)}}$ **acquisition**	No	224 (57.1%)	168 (42.9%)	295 (75.3%)	97 (24.7%)	339 (86.5%)	53 (13.5%)
	Yes	35 (23.6%)	113 (76.4%)	92 (62.2%)	56 (37.8%)	117 (79.1%)	31 (20.9%)
	p-value*	<0.001		<0.01		0.03	
qnrS	No	-	-	213 (82.2%)	46 (17.8%)	243 (93.8%)	16 (6.2%)
acquisition	Yes	-	-	174 (61.9%)	107 (38.1%)	213 (75.8%)	68 (24.2%)
	p-value*	-		<0.001		<0.001	
qnrB	No	-	-	-	-	342 (88.4%)	45 (11.6%)
acquisition	Yes	-	-	-	-	114 (74.5%)	39 (25.5%)
	p-value*	-		-		<0.001	

*Pearson chi-square test.

For every comparison between two AMR genes, travellers that contained either one of these AMR genes pre-travel were excluded from the analysis (N = 540).

Supplementary Table 4. Bivariable and multivariable logistic regression on potential predictors for bla$_{\text{CTX-M-(1, 2 and 9)}}$ acquisition among at risk intercontinental travellers in the final logistic regression model.

	Number of travellers (N = 637)§	Travellers with *bla*$_{\textit{CTX-M-(1, 2 and 9)}}$ gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Region visited						
Eastern Africa‡	119 (18.7%)	16/114 (14.0)	1.00		1.00	
Northern Africa	45 (7.1)	9/44 (20.5)	1.58 (0.64-3.89)	0.32	1.76 (0.67-4.60)	0.25
Southern Asia	127 (19.9%)	64/122 (52.5)	6.76 (3.58-12.78)	<0.001	8.38 (4.20-16.69)	<0.001
South-Eastern Asia	346 (54.3%)	80/333 (24.0)	1.94 (1.08-3.48)	0.03	1.72 (0.91-3.24)	0.09
Sex						
Male‡	295 (46.4%)	83/282 (29.4)	1.00			
Female	341 (53.6%)	86/330 (26.1)	0.80 (0.55-1.17)	0.25		
Age (median in years [IQR])	51.50 [34.94:60.48]	50.19 [29.11:58.02]	0.98 (0.97-1.00)	0.02		
BMI (median [IQR])	24.22 [22.04:26.42]	24.46 [22.21: 26.71]	1.02 (0.97-1.08)	0.45		
Country of birth						
Netherlands‡	595 (93.7%)	159/571 (27.8)	1.00			
Other country	40 (6.3%)	10/40 (25.0)	0.72 (0.33-1.56)	0.40		
Education level						
No education, elementary school or pre-vocational secondary education‡	71 (11.2%)	23/70 (32.9)	1.00			
Vocational secondary education or pre-university education	153 (24.2%)	37/146 (25.3)	0.71 (0.37-1.37)	0.31		
Higher professional education	216 (34.0%)	55/208 (26.4)	0.73 (0.39-1.34)	0.30		
Academic education	195 (30.7%)	54/187 (28.9)	0.83 (0.44-1.53)	0.54		

	Number of travellers (N = 637)§	Travellers with $bla_{CTX\text{-}M\text{-}(1,\ 2\ and\ 9)}$ gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Alcohol use (median, number of glasses per week [IQR])	5.00 [2.00:10.00]	6.00 [2.00:10.00]	1.01 (0.98-1.04)	0.65		
Cigarette smoking						
No‡	478 (79.7%)	127/464 (27.4)	1.00			
Yes	122 (20.3%)	29/114 (25.4)	0.95 (0.58-1.54	0.83		
Travel duration (median in days [IQR])	20.00 [15.00:23.00]	20.00 [15.00:25.00]	1.02 (1.00-1.03)	0.09		
Purpose of travel						
Holiday‡	556 (87.3%)	149/533 (28.0)	1.00			
Work/internship	33 (5.2%)	7/33 (21.2)	0.86 (0.34-2.16)	0.75		
Visit to family or friends	24 (3.8%)	7/24 (29.2)	1.37 (0.53-3.53)	0.52		
Other	24 (3.8%)	6/23 (26.1)	1.23 (0.44-3.46)	0.70		
Diarrhoea before travel						
No diarrhoea‡	404 (63.7%)	109/392 (27.8)	1.00			
Diarrhoea in the past three months	193 (30.4%)	53/184 (28.8)	1.06 (0.71-1.59)	0.77		
Diarrhoea at this moment only	3 (0.5%)	0/3 (0)	0	1.00		
Diarrhoea in the past three months and this moment	34 (5.4%)	7/31 (22.6)	0.75 (0.30-1.84)	0.53		
Fever within three months prior to travel						
No‡	582 (91.4%)	152/558 (27.2)	1.00			
Yes	55 (8.6%)	17/55 (30.9)	1.29 (0.69-2.44)	0.42		

Supplementary Table 4 *Continued*

	Number of travellers (N = 637)§	Travellers with *bla*$_{CTX\text{-}M\text{-}(1,\,2\text{ and }9)}$ gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Antibiotics use within three months prior to travel						
No‡	635 (99.7%)	167/611 (27.3)	1.00			
Yes	2 (0.3%)	2/2 (100)	>10	1.00		
Antacids use within three months prior to travel						
No‡	558 (88.0%)	143/538 (26.6)	1.00			
Yes	76 (12.0%)	26/72 (36.1)	1.44 (0.84-2.48)	0.19		
Medical history						
Chronic disease						
No ‡	500 (79.5%)	132/480 (26.6)	1.00			
Yes	129 (20.5%)	33/126 (26.2)	0.96 (0.60-1.52)	0.85		
Chronic bowel disease						
No‡	629 (98.7%)	167/605 (27.6)	1.00			
Yes	8 (1.3%)	2/8 (25.0)	1.26 (0.25-6.38)	0.78		
Probiotics use						
Hardly ever‡	489 (77.1%)	150/532 (28.2)	1.00			
Occasionally	71 (11.2%)	6/25 (24.0)	0.68 (0.37-1.27)	0.22		
Frequently	74 (11.7%)	3/11 (27.3)	0.70 (0.38-1.29)	0.25		
Daily patient contact						
No profession in healthcare‡	495 (78.2%)	128/478 (26.8)	1.00			
Profession in healthcare without daily patient contact	34 (5.4%)	10/33 (30.3)	1.25 (0.56-2.79)	0.59		

	Number of travellers (N = 637)§	Travellers with *bla*$_{CTX\text{-}M\text{-}(1,\ 2\ and\ 9)}$ gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Medical profession in healthcare with daily patient contact	59 (9.3%)	19/54 (35.2)	1.38 (0.74-2.58)	0.31		
Other profession in healthcare with daily patient contact	45 (7.1%)	11/44 (25.0)	0.97 (0.47-2.04)	0.95		
Diet						
No diet‡	572 (89.8%)	152/548 (27.7)	1.00			
Vegetarian or vegan	38 (6.0%)	10/38 (26.3)	0.84 (0.38-1.83)	0.65		
Other	27 (4.2%)	7/27 (25.9)	0.82 (0.33-2.08)	0.68		
During travel						
Antibiotics use during travel						
No‡	589 (93.8%)	142/565 (25.1)	1.00		1.00	
Yes	39 (6.2%)	23/39 (59.0)	5.37 (2.69-10.71)	<0.001	5.05 (1.93-13.15)	0.001
Travellers' diarrhoea¥						
No travellers' diarrhoea‡	382 (61.0%)	89/369 (24.1)	1.00		1.00	
Diarrhoea during travel	204 (32.6%)	63/194 (32.5)	1.65 (1.10-2.47)	0.02	1.56 (1.01-2.40)	0.04
Diarrhoea immediately after travel	9 (1.4%)	3/9 (33.3)	1.43 (0.32-6.49)	0.64	1.80 (0.39-8.34)	0.45
Diarrhoea during and immediately after travel	31 (5.0%)	13/30 (43.3)	2.74 (1.23-6.11)	0.01	2.84 (1.24-6.53)	0.01
Fever during travel						
No‡	594 (93.4%)	150/571 (26.3)	1.00			
Yes	42 (6.6%)	19/41 (46.3)	2.29 (1.17-4.49)	0.02		
Medical care during travel						
None‡	610 (95.8%)	154/586 (26.3)	1.00		1.00	
Visit to doctor or hospital	27 (4.2%)	15/27 (55.6)	4.05 (1.80-9.10)	0.001	1.35 (0.42-4.38)	0.62

Supplementary Table 4 *Continued*

	Number of travellers (N = 637)§	Travellers with *bla*$_{CTX\text{-}M\text{-}(1,\ 2\ and\ 9)}$ gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Use anti-diarrhoeal drugs during travel						
No‡	491 (77.1%)	123/471 (26.1)	1.00			
Yes	146 (22.9%)	46/142 (32.4)	1.56 (1.01-2.39)	0.04		
Use antacids during travel						
No‡	609 (95.6%)	160/586 (27.3)	1.00			
Yes	28 (4.4%)	9/27 (33.3)	1.33 (0.57-3.12)	0.51		
Use analgesics during travel						
No‡	531 (83.4%)	140/513 (27.4)	1.00			
Yes	106 (16.6%)	29/100 (29.0)	1.01 (0.62-1.66)	0.96		
Use other medication without prescription during travel						
No‡	574 (90.1%)	155/553 (28.0)	1.00			
Yes	63 (9.9%)	14/60 (23.3)	0.68 (0.35-1.31)	0.25		
Activities during travel						
Attendance large gathering						
No‡	592 (93.1%)	154/569 (27.1)	1.00			
Yes	44 (6.9%)	15/43 (34.9)	0.74 (0.36-1.51)	0.41		
Visit to local market						
Never ‡	56 (8.8%)	13/56 (23.2)	1.00			
Occasionally	491 (77.2%)	129/469 (27.5)	1.08 (0.54-2.15)	0.84		
Daily	89 (14.0%)	27/87 (31.0)	1.51 (0.67-3.41)	0.32		

	Number of travellers (N = 637)§	Travellers with *bla*$_{CTX\text{-}M\text{-}(1,\,2\text{ and }9)}$ gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Daily meal at local restaurant						
Never ‡	75 (11.8%)	16/74 (21.6)	1.00			
Occasionally	330 (51.9%)	79/314 (25.2)	0.97 (0.51-1.87)	0.93		
Daily	231 (36.3%)	74/224 (33.0)	1.46 (0.74-2.91)	0.28		
Meal at food stalls along the road						
Never ‡	403 (63.4%)	98/388 (25.3)	1.00			
Occasionally	199 (31.3%)	57/192 (29.7)	1.18 (0.78-1.77)	0.44		
Daily	34 (5.3%)	14/32 (43.8)	2.88 (1.34-6.19)	0.01		
Holiday type						
Backpacking						
No‡	471 (73.9%)	108/456 (23.7)	1.00		1.00	
Yes	166 (26.1%)	61/157 (38.9)	1.81 (1.20-2.72)	0.004	1.51 (0.96-2.37)	0.07
Beach						
No‡	469 (73.6%)	132/451 (29.3)	1.00			
Yes	168 (26.4%)	37/162 (22.8)	0.83 (0.54-1.29)	0.41		
Frequency of washing hands before meals during travel						
Never or sometimes washing with alcohol and/or soap‡	252 (39.6%)	74/243 (30.5)	1.00		1.00	
Most of the time washing with alcohol	63 (9.9%)	21/60 (35.0)	0.91 (0.48-1.73)	0.78	0.94 (0.48-1.83)	0.85
Most of the time washing with soap	232 (36.5%)	51/222 (23.0)	0.65 (0.42-1.01)	0.05	0.53 (0.33-0.85)	0.01
Most of the time washing with alcohol and soap	89 (14.0%)	23/87 (26.4)	0.66 (0.37-1.19)	0.17	0.59 (0.31-1.10)	0.10

Supplementary Table 4 *Continued*

	Number of travellers (N = 637)§	**Travellers with $bla_{CTX\text{-}M\text{-}(1,\ 2\ and\ 9}$) gene acquisition n/N (%)**	**Odds ratio (95% CI)***	**p-value**	**Adjusted Odds ratio (95% CI)****	**Adjusted p-value**
Frequency of washing hands directly after toilet use during travel						
Never or sometimes washing with alcohol and/or soap‡	123 (19.3%)	32/119 (26.9)	1.00			
Most of the time washing with alcohol	49 (7.7%)	10/48 (20.8)	0.64 (0.27-1.50)	0.30		
Most of the time washing with soap	362 (56.9%)	93/346 (26.9)	0.96 (0.59-1.58)	0.89		
Most of the time washing with alcohol and soap	102 (16.0%)	34/99 (34.3)	1.24 (0.68-2.28)	0.49		
Food consumption during travel						
Pork¶						
Hardly ever‡	356 (56.0%)	99/344 (28.8)	1.00			
Occasionally	219 (34.4%)	49/208 (23.6)	1.01 (0.66-1.57)	0.95		
Often	61 (9.6%)	21/60 (35.0)	2.03 (1.08-3.80)	0.03		
Chicken¶						
Hardly ever ‡	94 (14.8%)	31/91 (34.1)	1.00			
Occasionally	194 (30.5%)	47/186 (25.3)	0.94 (0.52-1.69)	0.83		
Often	348 (54.7%)	91/335 (27.2)	0.96 (0.56-1.65)	0.87		
Raw vegetables¶						
Hardly ever ‡	191 (30.0%)	44/184 (23.9)	1.00			
Occasionally	242 (38.1%)	69/235 (29.4)	1.45 (0.91-2.30)	0.12		
Often	203 (31.9%)	56/193 (29.0)	1.76 (1.07-2.87)	0.03		

	Number of travellers (N = 637)§	Travellers with $bla_{CTX\text{-}M\text{-}(1,\ 2\ and\ 9)}$ gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Unpeeled fruit¶						
Hardly ever ‡	448 (70.4%)	116/432 (26.9)	1.00			
Occasionally	111 (17.5%)	27/107 (25.2)	0.91 (0.55-1.51)	0.72		
Often	77 (12.1%)	26/73 (35.6)	1.51 (0.87-2.63)	0.14		
Shellfish¶						
Hardly ever‡	547 (86.0%)	140/527 (26.6)	1.00		1.00	
Occasionally	71 (11.2%)	21/67 (31.3)	1.48 (0.83-2.64)	0.19	1.77 (0.94-3.33)	0.08
Often	18 (2.8%)	8/18 (44.4)	3.27 (1.24-8.64)	0.02	3.81 (1.38-10.52)	0.01
Raw fish¶						
Hardly ever‡	603 (94.8)	156/580 (26.9)	1.00			
Occasionally and often	33 (5.2%)	13/32 (40.6)	2.46 (1.16-5.21)	0.02		

‡Reference category

§Some numbers do not add up to 637 due to missing data

*Only adjusted for region visited defined as: Eastern Africa, Northern Africa, Southern Asia and South-Eastern Asia

**Adjusted for region visited as well as sex, age, BMI, country of birth, education level, alcohol use, smoking, travel duration (in days), purpose of travel, diarrhoea, fever, antibiotic use, antacid use, chronic disease, bowel disease, probiotics use, daily patient contact, diet, travellers' diarrhoea, fever during travel, medical care during travel, use of medication without prescription (antidiarrheal drugs; analgesics; antacids), antibiotic use, activities during travel (attendance large (religious) gathering; visiting local markets, daily meal at local restaurant, meal at food stalls along the road, vacation type (backpacking; visit to the beach), daily hand washing before eating/after toilet use, pork, chicken, raw vegetables, unpeeled fruit, shellfish or raw fish

¥Travellers' diarrhoea was characterized as three or more loose stools within a 24-hour timeframe, with or without accompanying symptoms

¶Hardly ever = never or less than once a week, occasionally = approximately once a week, often = multiple times a week or daily

Supplementary Table 5. Bivariable and multivariable logistic regression on potential predictors for mcr-(1,3,4 and 5) gene acquisition among at risk intercontinental travellers in the final logistic regression model.

	Number of travellers (N = 473)§	**Travellers with *mcr-(1,3,4 and 5)* gene acquisition n/N (%)**	**Odds ratio (95% CI)***	**p-value**	**Adjusted Odds ratio (95% CI)****	**Adjusted p-value**
Region visited						
Southern Asia	127 (26.8%)	5/126 (4.0)	1.00		1.00	
South-Eastern Asia	346 (73.2%)	90/344 (26.2)	8.58 (3.40-21.65)	<0.001	8.33 (3.03-22.94)	<0.001
Sex						
Male‡	295 (46.7%)	38/219 (17.4)	1.00		1.00	
Female	341 (53.3%)	57/251 (22.7)	1.50 (0.93-2.40)	0.09	1.78 (1.01-3.15)	0.05
Age (median in years [IQR])	51.50 [33.87:60.40]	55.09 [39.73:62.97]	1.02 (1.01-1.04)	0.01	1.03 (1.00-1.05)	0.02
BMI (median [IQR])	24.22 [22.09:26.54]	24.07 [22.20:26.99]	0.99 (0.93-1.06)	0.82		
Country of birth						
Netherlands‡	439 (93.0%)	87/436 (20.0)	1.00			
Other country	33 (7.0%)	8/33 (24.2)	1.46 (0.61-3.50)	0.39		
Education level						
No education, elementary school or pre-vocational secondary education‡	54 (11.4%)	14/54 (25.9)	1.00		1.00	
Vocational secondary education or pre-university education	115 (24.4%)	18/114 (15.8)	0.51 (0.22-1.14)	0.10	0.50 (0.20-1.30)	0.16
Higher professional education	159 (33.7%)	39/158 (24.3)	0.95 (0.46-1.99)	0.89	0.89 (0.36-2.17)	0.80
Academic education	144 (30.5%)	24/143 (16.8)	0.58 (0.27-1.26)	0.17	0.93 (0.35-2.46)	0.88
Alcohol use (median, number of glasses per week [IQR])	6 [2:10]	6 [2:10]	1.01 (0.97-1.04)	0.74		

	Number of travellers (N = 473)§	Travellers with *mcr-(1,3,4 and 5)* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Cigarette smoking						
No‡	352 (79.3%)	67/349 (19.2)	1.00			
Yes	92 (20.7%)	14/92 (15.2)	0.70 (0.37-1.32)	0.27		
Travel duration (median in days [IQR])	20.00 [16:25]	21 [18:24]	0.99 (0.97-1.02)	0.62		
Purpose of travel						
Holiday‡	436 (92.2%)	88/433 (20.3)	1.00			
Work/internship	14 (3.0%)	0/14 (0)	0	1.00		
Visit to family or friends	15 (3.2%)	6/15 (40.0)	2.57 (0.85-7.82)	0.10		
Other	8 (1.7%)	1/8 (12.5)	0.79 (0.09-7.11)	0.83		
Diarrhoea before travel						
No diarrhoea‡	301 (63.9%)	62/298 (20.8)	1.00			
Diarrhoea in the past three months	140 (29.7%)	28/140 (20.0)	0.96 (0.57-1.61)	0.87		
Diarrhoea at this moment only	3 (0.6%)	1/3 (33.3)	1.35 (0.12-15.15)	0.81		
Diarrhoea in the past three months and this moment	27 (5.7%)	4/27 (14.8)	0.61 (0.20-1.85)	0.38		
Fever within three months prior to travel						
No‡	438 (92.6%)	86/435 (19.8)	1.00			
Yes	35 (7.4%)	9/35 (25.7)	1.48 (0.65-3.40)	0.35		
Antibiotics use within three months prior to travel						
No‡	471 (99.6%)	95/468 (20.3)	1.00			
Yes	2 (0.4%)	0/2 (0)	0	1.00		

Supplementary Table 5 *Continued*

	Number of travellers (N = 473)§	Travellers with *mcr-(1,3,4 and 5)* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Antacids use within three months prior to travel						
No‡	410 (87.2%)	80/408 (19.6)	1.00			
Yes	60 (12.8%)	14/59 (23.7)	1.35 (0.69-2.64)	0.39		
Medical history						
Chronic disease						
No ‡	366 (78.5%)	70/365 (19.2)	1.00			
Yes	100 (21.5%)	24/98 (24.5)	1.26 (0.73-2.18)	0.40		
Chronic bowel disease						
No‡	468 (98.9%)	94/465 (20.2)	1.00			
Yes	5 (1.1%)	1/5 (20.0)	0.70 (0.08-6.37)	0.75		
Probiotics use						
Hardly ever‡	362 (76.9%)	73/360 (20.3)	1.00		1.00	
Occasionally	52 (11.0%)	6/52 (11.5)	0.54 (0.22-1.34)	0.18	0.52 (0.17-1.54)	0.24
Frequently	57 (12.1%)	15/56 (26.8)	1.40 (0.72-2.73)	0.32	1.14 (0.50-2.59)	0.75
Daily patient contact						
No profession in healthcare‡	364 (77.6%)	70/362 (19.3)	1.00		1.00	
Profession in healthcare without daily patient contact	26 (5.6%)	4/26 (15.4)	0.71 (0.23-2.17)	0.55	0.77 (0.21-2.80)	0.69
Medical profession in healthcare with daily patient contact	47 (10.0%)	9/46 (19.6)	1.10 (0.50-2.47)	0.81	1.28 (0.47-3.44)	0.63
Other profession in healthcare with daily patient contact	32 (6.8%)	11/32 (34.4)	2.14 (0.95-4.82)	0.07	1.71 (0.58-5.04)	0.33

	Number of travellers (N = 473)§	Travellers with *mcr-(1,3,4 and 5)* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Diet						
No diet‡	424 (89.7%)	89/421 (21.1)	1.00			
Vegetarian or vegan	29 (6.1%)	6/29 (20.7)	1.05 (0.40-2.74)	0.93		
Other	20 (4.2%)	0/20 (0)	0	1.00		
During travel						
Antibiotics use during travel						
No‡	434 (93.1%)	80/432 (18.5)	1.00		1.00	
Yes	32 (6.9%)	15/31 (48.4)	3.44 (1.60-7.41)	0.002	3.42 (0.88-13.34)	0.08
Travellers' diarrhoea¥						
No travellers' diarrhoea‡	283 (61,1%)	51/280 (18.2)	1.00		1.00	
Diarrhoea during travel	154 (33.3%)	35/154 (22.7)	1.23 (0.75-2.03)	0.41	1.29 (0.72-2.32)	0.39
Diarrhoea immediately after travel	5 (1.1%)	1/5 (20.0)	2.05 (0.18-22.96)	0.56	2.98 (0.24-36.94)	0.40
Diarrhoea during and immediately after travel	21 (4.5%)	6/21 (28.6)	1.88 (0.66-5.35)	0.23	2.18 (0.61-7.80)	0.23
Fever during travel						
No‡	440 (93.%)	86/438 (19.6)	1.00			
Yes	33 (7.0%)	9/32 (28.1)	2.48 (0.95-6.47)	0.06	1.52 (0.45-5.08)	0.50
Medical care during travel						
None‡	452 (95.6%)	87/450 (19.6)	1.00		1.00	
Visit to doctor or hospital	21 (4.4%)	8/20 (40.0)	2.48 (0.95-6.47)	0.06	1.08 (0.19-6.23)	0.93
Use anti-diarrhoeal drugs during travel						
No‡	370 (78.2%)	72/367 (19.6)	1.00			
Yes	103 (21.8%)	23/103 (22.3)	0.31 (0.07-1.35)	0.12		

Supplementary Table 5 *Continued*

	Number of travellers (N = 473)§	Travellers with *mcr-(1,3,4 and 5)* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Use antacids during travel						
No‡	449 (94.9%)	93/447 (20.8)	1.00		1.00	
Yes	24 (5.1%)	2/23 (8.7)	0.61 (0.31-1.20)	0.15	0.19 (0.03-1.09)	0.06
Use analgesics during travel						
No‡	389 (82.2%)	84/389 (21.6)	1.00		1.00	
Yes	84 (17.8%)	12/84 (14.3)	0.75 (0.34-1.61)	0.15	0.67 (0.30-1.47)	0.32
Use other medication without prescription during travel						
No‡	419 (88.6%)	86/416 (20.7)	1.00			
Yes	54 (11.4%)	9/54 (16.7)	0.75 (0.34-1.61)	0.46		
Activities during travel						
Attendance large gathering						
No‡	429 (90.9%)	92/426 (21.6)	1.00		1.00	
Yes	43 (9.1%)	2/43 (4.7)	0.29 (0.07-1.27)	0.10	0.22 (0.04-1.10)	0.07
Visit to local market						
Never ‡	29 (6.1%)	3/29 (10.3)	1.00		1.00	
Occasionally	377 (79.9%)	75/374 (20.1)	2.07 (0.60-7.21)	0.25	1.60 (0.43-5.98)	0.48
Daily	66 (14.0%)	16/66 (24.2)	2.23 (0.58-8.58)	0.24	1.85 (0.43-7.93)	0.41
Daily meal at local restaurant						
Never ‡	29 (6.1%)	5/29 (17.2)	1.00			
Occasionally	235 (49.8%)	39/234 (16.7)	0.86 (0.30-2.50)	0.79		
Daily	208 (44.1%)	50/206 (24.3)	1.22 (0.42-3.50)	0.71		

	Number of travellers (N = 473)§	Travellers with *mcr-(1,3,4 and 5)* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Meal at food stalls along the road						
Never ‡	277 (58.7%)	53/276 (19.2)	1.00			
Occasionally	164 (34.7%)	31/162 (19.1)	0.96 (0.58-1.60)	0.89		
Daily	31 (6.6%)	10/31 (32.3)	1.45 (0.64-3.29)	0.38		
Holiday type						
Backpacking						
No‡	330 (69.8%)	68/328 (20.7)	1.00			
Yes	143 (30.2%)	27/142 (19.0)	1.00 (0.60-1.68)	1.00		
Beach						
No‡	344 (72.7%)	64/342 (18.7)	1.00			
Yes	198 (27.3%)	30/195 (24.2)	1.09 (0.66-1.80)	0.74		
Frequency of washing hands before meals during travel						
Never or sometimes washing with alcohol and/or soap‡	198 (41.9%)	30/195 (15.4)	1.00		1.00	
Most of the time washing with alcohol	49 (10.4%)	9/49 (18.4)	1.91 (0.80-4.55)	0.14	2.33 (0.64-8.46)	0.20
Most of the time washing with soap	158 (33.5%)	41/158 (25.9)	2.26 (1.31-3.89)	0.003	1.45 (0.72-2.90)	0.30
Most of the time washing with alcohol and soap	67 (14.2%)	14/67 (20.9)	2.10 (1.00-4.41)	0.05	1.32 (0.44-3.96)	0.62

Supplementary Table 5 *Continued*

	Number of travellers (N = 473)§	Travellers with *mcr-(1,3,4 and 5)* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Frequency of washing hands directly after toilet use during travel						
Never or sometimes washing with alcohol and/or soap‡	88 (18.6%)	10/86 (11.6)	1.00		1.00	
Most of the time washing with alcohol	30 (6.4%)	3/30 (10.0)	1.05 (0.26-4.21)	0.95	0.52 (0.08-3.50)	0.50
Most of the time washing with soap	273 (57.8%)	62/272 (22.8)	2.31 (1.11-4.80)	0.03	2.04 (0.78-5.33)	0.15
Most of the time washing with alcohol and soap	81 (17.2%)	19/81 (23.5)	2.85 (1.20-6.75)	0.02	2.09 (0.59-7.34)	0.25
Food consumption during travel						
Pork¶						
Hardly ever‡	250 (53.0%)	44/248 (17.7)	1.00			
Occasionally	170 (36.0%)	37/170 (21.8)	0.86 (0.51-1.43)	0.55		
Often	52 (11.0%)	13/51 (25.5)	0.93 (0.45-1.91)	0.83		
Chicken¶						
Hardly ever ‡	66 (14.0%)	11/66 (16.7)	1.00			
Occasionally	129 (27.3%)	26/129 (20.2)	0.68 (0.29-1.58)	0.37		
Often	277 (58.7%)	57/274 (20.8)	0.67 (0.31-1.47)	0.32		
Raw vegetables¶						
Hardly ever ‡	151 (32.0%)	24/151 (15.9)	1.00		1.00	
Occasionally	181 (38.3%)	45/179 (25.1)	1.81 (1.02-3.21)	0.04	1.88 (0.95-3.71)	0.07
Often	140 (29.7%)	25/139 (18.0)	0.96 (0.51-1.80)	0.89	1.13 (0.52-2.45)	0.75

	Number of travellers (N = 473)§	**Travellers with *mcr-(1,3,4 and 5)* gene acquisition n/N (%)**	**Odds ratio (95% CI)***	**p-value**	**Adjusted Odds ratio (95% CI)****	**Adjusted p-value**
Unpeeled fruit¶						
Hardly ever ‡	336 (71.2%)	69/334 (20.7)	1.00			
Occasionally	82 (17.4%)	18/81 (22.2)	1.13 (0.62-2.08)	0.69		
Often	54 (11.4%)	7/54 (13.0)	0.64 (0.27-1.51)	0.31		
Shellfish¶						
Hardly ever‡	393 (83.3%)	76/392 (19.4)	1.00		1.00	
Occasionally	65 (13.8%)	12/64 (18.8)	0.72 (0.36-1.44)	0.35	0.81 (0.35-1.83)	0.81
Often	14 (3.0%)	6/13 (46.2)	2.42 (0.79-7.44)	0.12	4.79 (1.26-18.22)	0.02
Raw fish¶						
Hardly ever‡	441 (93.4)	87/438 (19.9)	1.00			
Occasionally and often	31 (6.6%)	7/31 (22.6)	0.82 (0.34-1.97)	0.65		

‡Reference category

§Only included participants who travelled to Southern Asia or South-Eastern Asia due to low or no acquisition rate in other regions and some numbers may not add up to 473 due to missing data

*Only adjusted for region visited defined as: Southern Asia and South-Eastern Asia

**Adjusted for region visited as well as sex, age, BMI, country of birth, education level, alcohol use, smoking, travel duration (in days), purpose of travel, diarrhoea, fever, antibiotic use, antacid use, chronic disease, bowel disease, probiotics use, daily patient contact, diet, travellers' diarrhoea, fever during travel, medical care during travel, use of medication without prescription (antidiarrheal drugs; analgesics; antacids), antibiotic use, activities during travel (attendance large (religious) gathering; visiting local markets, daily meal at local restaurant, meal at food stalls along the road, vacation type (backpacking; visit to the beach), daily hand washing before eating/after toilet use, pork, chicken, raw vegetables, unpeeled fruit, shellfish or raw fish

¥Travellers' diarrhoea was characterized as three or more loose stools within a 24-hour timeframe, with or without accompanying symptoms

¶Hardly ever= never or less than once a week, occasionally= approximately once a week, often= multiple times a week or daily

Supplementary Table 6. Bivariable and multivariable logistic regression on potential predictors for qnrS gene acquisition among at risk intercontinental travellers in the final logistic regression model.

	Number of travellers (N = 637)§	Travellers with *qnrS* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Region visited						
Eastern Africa‡	119 (18.7%)	33/115 (28.7)	1.00		1.00	
Northern Africa	45 (7.1)	14/45 (31.1)	1.12 (0.53-2.37)	0.76	1.16 (0.52-2.63)	0.72
Southern Asia	127 (19.9%)	81/122 (66.4)	4.91 (2.83-8.52)	<0.001	4.58 (2.53-8.30)	<0.001
South-Eastern Asia	346 (54.3%)	193/333 (58.0)	3.43 (2.17-5.42)	<0.001	3.32 (2.00-5.53)	<0.001
Sex						
Male‡	295 (46.4%)	150/285 (52.6)	1.00			
Female	341 (53.6%)	171/329 (52.0)	0.97 (0.70-1.35)	0.86		
Age (median in years [IQR])	51.50 [34.94:60.48]	51.60 [35.62:59.70]	1.00 (0.99-1.01)	0.69		
BMI (median [IQR])	24.22 [22.04:26.42]	24.44 [26.86: 24.22]	1.02 (0.97-1.07)	0.52		
Country of birth						
Netherlands‡	595 (93.7%)	300/574 (52.3)	1.00			
Other country	40 (6.3%)	21/39 (53.8)	0.94 (0.48-1.84)	0.86		
Education level						
No education, elementary school or pre-vocational secondary education‡	71 (11.2%)	40/69 (58.0)	1.00			
Vocational secondary education or pre-university education	153 (24.2%)	80/149 (53.7)	0.85 (0.47-1.55)	0.60		
Higher professional education	216 (34.0%)	106/209 (50.7)	0.75 (0.42-1.33)	0.33		
Academic education	195 (30.7%)	95/186 (51.1)	0.75 (0.42-1.35)	0.34		
Alcohol use (median, number of glasses per week [IQR])	5.00 [2.00:10.00]	5.00 [2.00:10.00]	0.97 (0.95-1.00)	0.04		

	Number of travellers (N = 637)§	Travellers with *qnrS* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Cigarette smoking						
No‡	478 (79.7%)	252/460 (54.8)	1.00		1.00	
Yes	122 (20.3%)	47/119 (39.5)	0.50 (0.33-0.77)	0.001	0.49 (0.32-0.78)	0.002
Travel duration (median in days [IQR])	20.00 [15.00:23.00]	20.00 [16.00:24.00]	1.00 (0.98-1.02)	0.92		
Purpose of travel						
Holiday‡	556 (87.3%)	289/537 (53.8)	1.00			
Work/internship	33 (5.2%)	9/32 (28.2)	0.46 (0.20-1.06)	0.07		
Visit to family or friends	24 (3.8%)	15/22 (68.2)	2.40 (0.92-6.31)	0.08		
Other	24 (3.8%)	8/24 (33.3)	0.69 (0.27-1.76)	0.44		
Diarrhoea before travel						
No diarrhoea‡	404 (63.7%)	211/388 (54.4)	1.00			
Diarrhoea in the past three months	193 (30.4%)	91/188 (48.4)	0.79 (0.55-1.13)	0.20		
Diarrhoea at this moment only	3 (0.5%)	1/3 (33.3)	0.33 (0.03-3.66)	0.37		
Diarrhoea in the past three months and this moment	34 (5.4%)	18/33 (54.5)	0.91 (0.44-1.91)	0.81		
Fever within three months prior to travel						
No‡	582 (91.4%)	296/561 (52.8)	1.00			
Yes	55 (8.6%)	25/54 (46.3)	0.87 (0.48-1.57)	0.65		
Antibiotics use within three months prior to travel						
No‡	635 (99.7%)	319/613 (52.0)	1.00			
Yes	2 (0.3%)	2/2 (100)	>10			

Supplementary Table 6 *Continued*

	Number of travellers (N = 637)§	Travellers with *qnrS* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Antacids use within three months prior to travel						
No‡	558 (88.0%)	280/540 (51.9)	1.00			
Yes	76 (12.0%)	40/73 (54.8)	1.05 (0.63-1.75)	0.85		
Medical history						
Chronic disease						
No ‡	500 (79.5%)	239/484 (49.4)	1.00		1.00	
Yes	129 (20.5%)	76/124 (61.3)	1.61 (1.05-2.44)	0.03	1.71 (1.08-2.71)	0.02
Chronic bowel disease						
No‡	629 (98.7%)	315/607 (51.9)	1.00			
Yes	8 (1.3%)	6/8 (75.0)	3.78 (0.71-20.07)	0.12		
Probiotics use						
Hardly ever‡	489 (77.1%)	245/471 (52.0)	1.00			
Occasionally	71 (11.2%)	35/69 (52.0)	0.91 (0.54-1.54)	0.72		
Frequently	74 (11.7%)	40/72 (55.6)	1.12 (0.67-1.89)	0.66		
Daily patient contact						
No profession in healthcare‡	495 (78.2%)	238/478 (49.8)	1.00		1.00	
Profession in healthcare without daily patient contact	34 (5.4%)	16/33 (48.5)	0.89 (0.43-1.85)	0.76	0.74 (0.34-1.60)	0.45
Medical profession in healthcare with daily patient contact	59 (9.3%)	38/56 (67.9)	2.09 (1.13-3.87)	0.02	2.26 (1.17-4.35)	0.02
Other profession in healthcare with daily patient contact	45 (7.1%)	28/44 (63.6)	1.95 (1.00-3.82)	0.05	1.65 (0.80-3.39)	0.18

	Number of travellers (N = 637)§	Travellers with *qnrS* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Diet						
No diet‡	572 (89.8%)	295/553 (53.3)	1.00		1.00	
Vegetarian or vegan	38 (6.0%)	16/37 (43.2)	0.61 (0.31-1.23)	0.17	0.36 (0.15-0.87)	0.02
Other	27 (4.2%)	10/25 (40.0)	0.55 (0.24-1.29)	0.17	0.35 (0.12-1.00)	0.05
During travel						
Antibiotics use during travel						
No‡	589 (93.8%)	294/570 (51.6)	1.00			
Yes	39 (6.2%)	23/36 (63.9)	1.68 (0.81-3.47)	0.16		
Travellers' diarrhoea¥						
No travellers' diarrhoea‡	382 (61.0%)	195/370 (52.7)	1.00			
Diarrhoea during travel	204 (32.6%)	98/194 (50.5)	0.90 (0.63-1.30)	0.58		
Diarrhoea immediately after travel	9 (1.4%)	4/9 (44.4)	0.83 (0.20-3.43)	0.80		
Diarrhoea during and immediately after travel	31 (5.0%)	20/31 (64.5)	1.86 (0.84-4.16)	0.13		
Fever during travel						
No‡	594 (93.4%)	299/573 (52.2)	1.00			
Yes	42 (6.6%)	21/41 (51.2)	0.89 (0.46-1.72)	0.73		
Medical care during travel						
None‡	610 (95.8%)	305/589 (51.8)	1.00			
Visit to doctor or hospital	27 (4.2%)	16/26 (61.5)	1.52 (0.66-3.51)	0.33		
Use anti-diarrhoeal drugs during travel						
No‡	491 (77.1%)	252/474 (53.2)	1.00			
Yes	146 (22.9%)	69/141 (48.9)	0.91 (0.62-1.35)	0.64		

Supplementary Table 6 *Continued*

	Number of travellers (N = 637)§	Travellers with *qnrS* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Use antacids during travel						
No‡	609 (95.6%)	307/587 (52.3)	1.00			
Yes	28 (4.4%)	14/28 (50.0)	0.79 (0.36-1.72)	0.55		
Use analgesics during travel						
No‡	531 (83.4%)	265/513 (51.7)	1.00			
Yes	106 (16.6%)	56/102 (54.9)	1.04 (0.67-1.62)	0.86		
Use other medication without prescription during travel						
No‡	574 (90.1%)	283/555 (51.0)	1.00			
Yes	63 (9.9%)	38/60 (63.3)	1.41 (0.80-2.48)	0.24		
Activities during travel						
Attendance large gathering						
No‡	592 (93.1%)	297/572 (51.9)	1.00			
Yes	44 (6.9%)	23/42 (54.8)	0.69 (0.36-1.35)	0.28		
Visit to local market						
Never ‡	56 (8.8%)	23/52 (44.2)	1.00			
Occasionally	491 (77.2%)	245/476 (51.5)	0.95 (0.51-1.77)	0.88		
Daily	89 (14.0%)	52/86 (60.5)	1.51 (0.72-3.17)	0.28		
Daily meal at local restaurant						
Never ‡	75 (11.8%)	29/73 (39.7)	1.00			
Occasionally	330 (51.9%)	154/317 (48.6)	0.99 (0.57-1.74)	0.99		
Daily	231 (36.3%)	137/224 (61.2)	1.39 (0.76-2.54)	0.28		

	Number of travellers (N = 637)§	Travellers with *qnrS* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Meal at food stalls along the road						
Never ‡	403 (63.4%)	186/386 (48.2)	1.00		1.00	
Occasionally	199 (31.3%)	109/195 (55.9)	1.17 (0.81-1.68)	0.40	1.23 (0.83-1.82)	0.31
Daily	34 (5.3%)	25/33 (75.8)	2.82 (1.21-6.53)	0.02	3.02 (1.25-7.32)	0.01
Holiday type						
Backpacking						
No‡	471 (73.9%)	227/457 (49.7)	1.00			
Yes	166 (26.1%)	94/158 (59.5)	1.20 (0.82-1.76)	0.35		
Beach						
No‡	469 (73.6%)	243/456 (53.3)	1.00			
Yes	168 (26.4%)	78/159 (49.1)	0.84 (0.57-1.23)	0.37		
Frequency of washing hands before meals during travel						
Never or sometimes washing with alcohol and/or soap‡	252 (39.6%)	127/244 (52.0)	1.00			
Most of the time washing with alcohol	63 (9.9%)	37/62 (59.7)	1.31 (0.72-2.38)	0.38		
Most of the time washing with soap	232 (36.5%)	107/222 (48.2)	0.94 (0.64-1.37)	0.73		
Most of the time washing with alcohol and soap	89 (14.0%)	49/86 (57.0)	1.23 (0.73-2.08)	0.43		

Supplementary Table 6 *Continued*

	Number of travellers (N = 637)§	Travellers with *qnrS* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Frequency of washing hands directly after toilet use during travel						
Never or sometimes washing with alcohol and/or soap‡	123 (19.3%)	56/118 (47.5)	1.00			
Most of the time washing with alcohol	49 (7.7%)	24/49 (49.0)	1.17 (0.58-2.37)	0.65		
Most of the time washing with soap	362 (56.9%)	180/349 (51.6)	1.13 (0.73-1.74)	0.59		
Most of the time washing with alcohol and soap	102 (16.0%)	60/98 (61.2)	1.60 (0.91-2.83)	0.10		
Food consumption during travel						
Pork¶						
Hardly ever‡	356 (56.0%)	175/346 (50.6)	1.00			
Occasionally	219 (34.4%)	108/207 (52.2)	1.07 (0.73-1.55)	0.74		
Often	61 (9.6%)	37/61 (60.7)	1.46 (0.81-2.64)	0.21		
Chicken¶						
Hardly ever ‡	94 (14.8%)	55/93 (59.1)	1.00		1.00	
Occasionally	194 (30.5%)	83/180 (46.1)	0.65 (0.38-1.11)	0.12	0.53 (0.27-1.03)	0.06
Often	348 (54.7%)	182/341 (53.4)	0.73 (0.44-1.21)	0.22	0.57 (0.30-1.08)	0.08
Raw vegetables¶						
Hardly ever ‡	191 (30.0%)	95/183 (51.9)	1.00			
Occasionally	242 (38.1%)	128/233 (54.9)	1.20 (0.80-1.80)	0.38		
Often	203 (31.9%)	97/198 (49.0)	1.03 (0.68-1.57)	0.89		

	Number of travellers (N = 637)§	Travellers with *qnrS* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	Adjusted p-value
Unpeeled fruit¶						
Hardly ever ‡	448 (70.4%)	220/434 (50.7)	1.00		1.00	
Occasionally	111 (17.5%)	66/108 (61.1)	1.60 (1.02-2.50)	0.04	1.79 (1.09-2.94)	0.02
Often	77 (12.1%)	34/72 (47.2)	0.89 (0.53-1.50)	0.66	0.97 (0.55-1.69)	0.90
Shellfish¶						
Hardly ever‡	547 (86.0%)	272/529 (51.4)	1.00			
Occasionally	71 (11.2%)	36/67 (53.7)	0.92 (0.54-1.56)	0.75		
Often	18 (2.8%)	12/18 (66.7)	2.01 (0.71-5.66)	0.19		
Raw fish¶						
Hardly ever‡	603 (94.8)	302/583 (51.8)	1.00			
Occasionally and often	33 (5.2%)	18/31 (58.1)	1.10 (0.52-2.34)	0.80		

‡Reference category

§Some numbers do not add up to 637 due to missing data

*Only adjusted for region visited defined as: Eastern Africa, Northern Africa, Southern Asia and South-Eastern Asia

**Adjusted for region visited as well as sex, age, BMI, country of birth, education level, alcohol use, smoking, travel duration (in days), purpose of travel, diarrhoea, fever, antibiotic use, antacid use, chronic disease, bowel disease, probiotics use, daily patient contact, diet, travellers' diarrhoea, fever during travel, medical care during travel, use of medication without prescription (antidiarrheal drugs; analgesics; antacids), antibiotic use, activities during travel (attendance large (religious) gathering; visiting local markets, daily meal at local restaurant, meal at food stalls along the road, vacation type (backpacking; visit to the beach), daily hand washing before eating/after toilet use, pork, chicken, raw vegetables, unpeeled fruit, shellfish or raw fish

¥Travellers' diarrhoea was characterized as three or more loose stools within a 24-hour timeframe, with or without accompanying symptoms

¶Hardly ever= never or less than once a week, occasionally= approximately once a week, often= multiple times a week or daily

Supplementary table 7. Bivariable and multivariable logistic regression on potential predictors for qnrB gene acquisition among at risk intercontinental travellers in the final logistic regression model.

	Number of travellers (N = 637)§	**Travellers with *qnrB* gene acquisition n/N (%)**	**Odds ratio (95% CI)***	**p-value**	**Adjusted Odds ratio (95% CI)****	**p-value**
Region visited						
Eastern Africa‡	119 (18.7%)	27/109 (24.8)	1.00		1.00	
Northern Africa	45 (7.1)	12/40 (30.0)	1.30 (0.58-2.91)	0.52	1.14 (0.50-2.59)	0.75
Southern Asia	127 (19.9%)	41/115 (35.7)	1.68 (0.94-3.00)	0.08	1.93 (1.06-3.53)	0.03
South-Eastern Asia	346 (54.3%)	85/310 (27.4)	1.15 (0.70-1.89)	0.59	1.09 (0.65-1.81)	0.75
Sex						
Male‡	295 (46.4%)	68/270 (25.2)	1.00			
Female	341 (53.6%)	97/303 (32.0)	1.39 (0.96-2.00)	0.08		
Age (median in years [IQR])	51.50 [34.94:60.48]	38.68 [59.92:51.60]	1.01 (0.99-1.02)	0.40		
BMI (median [IQR])	24.22 [22.04:26.42]	24.01 [22.11:26.53]	1.00 (0.94-1.05)	0.87		
Country of birth						
Netherlands‡	595 (93.7%)	154/537 (28.7)	1.00			
Other country	40 (6.3%)	11/36 (30.6)	1.05 (0.50-2.19)	0.90		
Education level						
No education, elementary school or pre-vocational secondary education‡	71 (11.2%)	23/63 (36.5)	1.00			
Vocational secondary education or pre-university education	153 (24.2%)	44/135 (32.6)	0.86 (0.46-1.62)	0.64		
Higher professional education	216 (34.0%)	47/197 (23.9)	0.54 (0.29-1.00)	0.05		
Academic education	195 (30.7%)	51/178 (28.7)	0.69 (0.37-1.27)	0.24		

	Number of travellers (N = 637)§	Travellers with *qnrB* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	p-value
Alcohol use (median, number of glasses per week [IQR])	5.00 [2.00:10.00]	5.00 [2.00:8.00]	0.98 (0.95-1.01)	0.12		
Cigarette smoking						
No‡	478 (79.7%)	133/426 (31.2)	1.00			
Yes	122 (20.3%)	27/116 (23.3)	0.67 (0.41-1.08)	0.1		
Travel duration (median in days [IQR])	20.00 [15.00:23.00]	19:00 [15.00:22.00]	0.99 (0.97-1.01)	0.26		
Purpose of travel						
Holiday‡	556 (87.3%)	148/501 (29.5)	1.00			
Work/internship	33 (5.2%)	8/30 (26.7)	0.88 (0.38-2.06)	0.77		
Visit to family or friends	24 (3.8%)	7/22 (31.8)	1.17 (0.46-2.97)	0.74		
Other	24 (3.8%)	2/21 (9.5)	0.26 (0.06-1.15)	0.08		
Diarrhoea before travel						
No diarrhoea‡	404 (63.7%)	111/363 (30.6)	1.00			
Diarrhoea in the past three months	193 (30.4%)	40/174 (23.0)	0.68 (0.44-1.03)	0.07		
Diarrhoea at this moment only	3 (0.5%)	1/3 (33.3)	1.23 (0.11-13.82)	0.87		
Diarrhoea in the past three months and this moment	34 (5.4%)	12/32 (37.5)	1.37 (0.64-2.92)	0.41		
Fever within three months prior to travel						
No‡	582 (91.4%)	147/521 (28.2)	1.00			
Yes	55 (8.6%)	18/53 (34.0)	1.31 (0.71-2.39)	0.39		

Supplementary Table 7 *Continued*

	Number of travellers (N = 637)§	Travellers with *qnrB* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	p-value
Antibiotics use within three months prior to travel						
No‡	635 (99.7%)	164/573 (28.6)	1.00			
Yes	2 (0.3%)	1/1 (100)	>10	1.00		
Antacids use within three months prior to travel						
No‡	558 (88.0%)	136/505 (26.9)	1.00		1.00	
Yes	76 (12.0%)	29/67 (43.3)	2.03 (1.20-3.43)	0.01	1.79 (1.05-3.06)	0.03
Medical history						
Chronic disease						
No ‡	500 (79.5%)	117/447 (26.2)	1.00		1.00	
Yes	129 (20.5%)	46/120 (38.3)	1.78 (1.16-2.73)	0.01	1.65 (1.06-2.57)	0.03
Chronic bowel disease						
No‡	629 (98.7%)	163/566 (28.8)	1.00			
Yes	8 (1.3%)	2/8 (25.0)	0.90 (0.18-4.50)	0.89		
Probiotics use						
Hardly ever‡	489 (77.1%)	128/436 (29.4)	1.00			
Occasionally	71 (11.2%)	18/66 (27.3)	0.88 (0.49-1.58)	0.68		
Frequently	74 (11.7%)	19/69 (27.5)	0.91 (0.51-1.60)	0.74		
Daily patient contact						
No profession in healthcare‡	495 (78.2%)	124/450 (27.6)	1.00			
Profession in healthcare without daily patient contact	34 (5.4%)	7/33 (21.2)	0.72 (0.30-1.70)	0.45		

	Number of travellers (N = 637)§	Travellers with *qnrB* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	p-value
Medical profession in healthcare with daily patient contact	59 (9.3%)	19/49 (38.8)	1.62 (0.87-3.00)	0.13		
Other profession in healthcare with daily patient contact	45 (7.1%)	15/39 (38.5)	1.69 (0.85-3.33)	0.13		
Diet						
No diet‡	572 (89.8%)	150/515 (29.1)	1.00			
Vegetarian or vegan	38 (6.0%)	10/34 (29.4)	0.97 (0.45-2.09)	0.94		
Other	27 (4.2%)	5/25 (20.0)	0.59 (0.22-1.61)	0.30		
During travel						
Antibiotics use during travel						
No‡	589 (93.8%)	152/528 (28.8)	1.00			
Yes	39 (6.2%)	12/38 (31.6)	1.19 (0.58-2.43)	0.64		
Travellers' diarrhoea¥						
No travellers' diarrhoea‡	382 (61.0%)	100/345 (29.0)	1.00			
Diarrhoea during travel	204 (32.6%)	51/183 (27.9)	0.96 (0.64-1.43)	0.84		
Diarrhoea immediately after travel	9 (1.4%)	2/9 (22.2)	0.66 (0.13-3.25)	0.60		
Diarrhoea during and immediately after travel	31 (5.0%)	12/27 (44.4)	1.99 (0.90-4.42)	0.09		
Fever during travel						
No‡	594 (93.4%)	152/536 (28.4)	1.00			
Yes	42 (6.6%)	13/37 (35.1)	1.31 (0.65-2.65)	0.46		
Medical care during travel						
None‡	610 (95.8%)	156/548 (28.5)	1.00			
Visit to doctor or hospital	27 (4.2%)	9/26 (34.6)	1.35 (0.59-3.09)	0.49		

Supplementary Table 7 *Continued*

	Number of travellers (N = 637)§	Travellers with *qnrB* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	p-value
Use anti-diarrhoeal drugs during travel						
No‡	491 (77.1%)	132/441 (29.9)	1.00			
Yes	146 (22.9%)	33/133 (24.8)	0.79 (0.51-1.24)	0.31		
Use antacids during travel						
No‡	609 (95.6%)	154/549 (28.1)	1.00			
Yes	28 (4.4%)	11/25 (44.0)	2.05 (0.91-4.64)	0.08		
Use analgesics during travel						
No‡	531 (83.4%)	144/483 (29.8)	1.00			
Yes	106 (16.6%)	21/91 (23.1)	0.68 (0.40-1.15)	0.15		
Use other medication without prescription during travel						
No‡	574 (90.1%)	151/524 (28.8)	1.00			
Yes	63 (9.9%)	14/50 (28.0)	0.97 (0.51-1.87)	0.94		
Activities during travel						
Attendance large gathering						
No‡	592 (93.1%)	161/539 (29.9)	1.00		1.00	
Yes	44 (6.9%)	3/34 (8.8)	0.18 (0.05-0.60)	0.01	0.19 (0.06-0.66)	0.01
Visit to local market						
Never ‡	56 (8.8%)	12/52 (23.1)	1.00			
Occasionally	491 (77.2%)	128/443 (28.9)	1.32 (0.67-2.64)	0.42		
Daily	89 (14.0%)	24/78 (30.8)	1.53 (0.67-3.46)	0.31		

	Number of travellers (N = 637)§	Travellers with *qnrB* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	p-value
Daily meal at local restaurant						
Never ‡	75 (11.8%)	23/69 (33.3)	1.00			
Occasionally	330 (51.9%)	87/300 (29.0)	0.76 (0.42-1.35)	0.34		
Daily	231 (36.3%)	54.204 (26.5)	0.65 (0.35-1.23)	0.19		
Meal at food stalls along the road						
Never ‡	403 (63.4%)	99/369 (26.8)	1.00			
Occasionally	199 (31.3%)	56/176 (31.8)	1.26 (0.85-1.88)	0.26		
Daily	34 (5.3%)	9/28 (32.1)	1.40 (0.60-3.26)	0.44		
Holiday type						
Backpacking						
No‡	471 (73.9%)	117/428 (27.3)	1.00			
Yes	166 (26.1%)	48/146 (32.9)	1.26 (0.83-1.90)	0.28		
Beach						
No‡	469 (73.6%)	119/421 (28.3)	1.00			
Yes	168 (26.4%)	46/153 (30.1)	1.15 (0.76-1.74)	0.51		
Frequency of washing hands before meals during travel						
Never or sometimes washing with alcohol and/or soap‡	252 (39.6%)	64/227 (28.2)	1.00			
Most of the time washing with alcohol	63 (9.9%)	20/56 (35.7)	1.31 (0.70-2.45)	0.40		
Most of the time washing with soap	232 (36.5%)	63/214 (29.4)	1.06 (0.70-1.60)	0.80		
Most of the time washing with alcohol and soap	89 (14.0%)	17/76 (22.4)	0.69 (0.37-1.28)	0.24		

Supplementary Table 7 *Continued*

	Number of travellers (N = 637)§	Travellers with *qnrB* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	p-value
Frequency of washing hands directly after toilet use during travel						
Never or sometimes washing with alcohol and/or soap‡	123 (19.3%)	27/114 (23.7)	1.00			
Most of the time washing with alcohol	49 (7.7%)	14/45 (31.1)	1.41 (0.65-3.04)	0.39		
Most of the time washing with soap	362 (56.9%)	100/323 (31.0)	1.44 (0.88-2.35)	0.15		
Most of the time washing with alcohol and soap	102 (16.0%)	23/91 (25.3)	1.04 (0.54-1.97)	0.92		
Food consumption during travel						
Pork¶						
Hardly ever‡	356 (56.0%)	97/319 (30.4)	1.00			
Occasionally	219 (34.4%)	58/199 (29.1)	1.01 (0.67-1.52)	0.97		
Often	61 (9.6%)	9/55 (16.4)	0.49 (0.22-1.06)	0.07		
Chicken¶						
Hardly ever ‡	94 (14.8%)	30/86 (34.9)	1.00			
Occasionally	194 (30.5%)	44/177 (24.9)	0.68 (0.38-1.21)	0.19		
Often	348 (54.7%)	90/310 (29.0)	0.83 (0.49-1.41)	0.49		
Raw vegetables¶						
Hardly ever ‡	191 (30.0%)	56/178 (31.5)	1.00			
Occasionally	242 (38.1%)	68/215 (31.6)	1.02 (0.66-1.56)	0.93		
Often	203 (31.9%)	40/180 (22.2)	0.65 (0.40-1.05)	0.08		

	Number of travellers (N = 637)§	Travellers with *qnrB* gene acquisition n/N (%)	Odds ratio (95% CI)*	p-value	Adjusted Odds ratio (95% CI)**	p-value
Unpeeled fruit¶						
Hardly ever ‡	448 (70.4%)	125/406 (30.8)	1.00			
Occasionally	111 (17.5%)	22/99 (22.2)	0.64 (0.38-1.08)	0.09		
Often	77 (12.1%)	17/68 (25.0)	0.72 (0.40-1.30)	0.28		
Shellfish¶						
Hardly ever‡	547 (86.0%)	147/495 (29.7)	1.00			
Occasionally	71 (11.2%)	16/61 (26.2)	0.87 (0.47-1.61)	0.66		
Often	18 (2.8%)	1/17 (5.9)	0.16 (0.02-1.24)	0.08		
Raw fish¶						
Hardly ever‡	603 (94.8)	154/540 (28.5)	1.00			
Occasionally and often	33 (5.2%)	10/33 (30.2)	1.19 (0.55-2.61)	0.66		

‡Reference category

§Some numbers do not add up to 637 due to missing data

*Only adjusted for region visited defined as: Eastern Africa, Northern Africa, Southern Asia and South-Eastern Asia

**Adjusted for region visited as well as sex, age, BMI, country of birth, education level, alcohol use, smoking, travel duration (in days), purpose of travel, diarrhoea, fever, antibiotic use, antacid use, chronic disease, bowel disease, probiotics use, daily patient contact, diet, travellers' diarrhoea, fever during travel, medical care during travel, use of medication without prescription (antidiarrheal drugs; analgesics; antacids), antibiotic use, activities during travel (attendance large (religious) gathering; visiting local markets, daily meal at local restaurant, meal at food stalls along the road, vacation type (backpacking; visit to the beach), daily hand washing before eating/after toilet use, pork, chicken, raw vegetables, unpeeled fruit, shellfish or raw fish

¥Travellers' diarrhoea was characterized as three or more loose stools within a 24-hour timeframe, with or without accompanying symptoms

¶Hardly ever= never or less than once a week, occasionally= approximately once a week, often= multiple times a week or daily

CHAPTER 6

Daily sampling reveals rapid microbiota alterations and antimicrobial resistance gene acquisition during intercontinental travel

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Abstract

With increasing global travel, individuals are frequently exposed to new diets, environments, and microbial communities that may influence gut microbiota dynamics and facilitate the acquisition of antimicrobial resistance genes (ARGs). This study aimed to elucidate the short-term effects of travel on gut microbiome dynamics and ARG acquisition using a high-frequency sampling approach. A cohort of eleven Dutch travellers to Asia self-collected 254 faecal swabs before, during, and after travel for microbiota and resistome profiling. Samples were analysed using qPCR targeting clinically relevant ARGs (*qnrB, qnrS* and $bla_{\text{CTX-M}}$) and profiled by 16S rRNA gene amplicon sequencing. Longitudinal analyses revealed pronounced inter- and intra-individual variation, with rapid shifts in microbiota composition observed within the first days of travel. An increase in Enterobacterales and a decline in commensal taxa were detected during early travel, coinciding with swift ARG acquisition. These findings underscore the key role of travel in global ARG dissemination.

Introduction

In our modern world, the increase in accessibility of global travel offers opportunities for cultural exchange, business opportunities as well as leisure activities. However, this heightened mobility also presents unique public health challenges, as increased mobility facilitates exposure to, and the transboundary spread of, geographically restricted pathogens as well as antimicrobial resistant microbes. *The most common health consequence of such exposures is the onset of gastrointestinal infections1.*

Beyond acute infections, growing attention has been given to the role of the gut microbiota in maintaining gut health, and by extension the overall well-being[2-4]. Disruptions in the gut microbiota can not only accompany symptoms but also serve as early indicators of susceptibility to various diseases[5-8]. A healthy adult gut microbiota is resilient to perturbations and stable over years, although slight day-to-day variations have been observed and major dietary shifts and lifestyle changes have the potential to alter the microbiota[9-11]. Such shifts potentially readily happen during travel to foreign countries due to the exposure to unfamiliar cuisines, environments, microbial exposures or complications such as traveller's diarrhoea.

Several studies have examined the impact of international travel on the gut microbiota[12-18]. However, most of the comparisons in these studies were performed between pre-travel and post-travel observations, with only a couple of studies investigating the temporal microbiota variability during travel. A case study on an individual who alternated a two-month stay in Italy with a two-month stay in Nigeria demonstrated that short-term geographical changes could result in shifts in gut microbiota structure[12]. Boolchandani et al.[13] observed within a cohort of 159 international students traveling to Peru that diarrhoea disrupted the stability of the taxonomic diversity. Nonetheless, results of both studies were based on weekly sampling intervals, which may not adequately capture the rapid shifts and day-to-day variability of the gut microbiota.

Moreover, intercontinental travel also serves as a potential vector for the dissemination of antimicrobial resistance genes (ARG). Previous research has shown that human travel to areas with a high prevalence of antimicrobial resistance (AMR) contributes to the spread of AMR bacteria or ARGs across geographic areas[19]. Yet, there are currently no published studies that characterise day-to-day changes in the resistome in the context of travel. Here, we collected faecal swabs at high-resolution from 11 intercontinental travellers taken before, daily during, and at several time-points after their journeys to investigate whether and how travel alters the composition of the gut microbiota. In addition, we screened faecal as well as skin microbiomes for several ARGs to assess the rate of ARG acquisition.

Results

In total, we investigated 11 travelling individuals, 6 female and 5 males. Travellers (Tr) 1 and 4 are the same individual who underwent two separate trips in different years (Table 1). The median duration of travel was 14 (IQR 11-22) days. All travellers visited countries in Asia. (Table 1). Furthermore, Tr1 and Tr2 were companions who travelled to the same country. Tr8, Tr9, Tr11 and Tr12 were a family a four who travelled together to Malaysia. None of the participants were hospitalized and they all reported that they did not use antibiotics during, or in the 3 months preceding travel. Both Tr1 and Tr11 suffered from a diarrheal episode during their travel.

Table 1: Characteristics of participants

Traveller (Tr) #	Sex	Age	Destination	Duration (days)	Diarrhoea during travel
1	M	24	South Korea	12	Yes, day 10
2	F	24	South Korea	12	No
3	M	37	India	21	No
4	M	24	India	10	No
5	F	38	China	21	No
7	M	25	Philippines	14	No
8	F	7	Malaysia	14	No
9	F	10	Malaysia	23	No
11	M	40	Malaysia	23	Yes, days 15 & 16
12	F	38	Malaysia	23	No
13	M	51	India	5	No
14	F	42	India	5	No

Transient and individual-specific changes in gut microbial diversity during and after travel

Altogether, 254 faecal swabs (33 pre-travel, 142 during travel and 79 post-travel) from twelve travel episodes, involving eleven individual participants, were subjected to DNA extraction, 16S rRNA gene amplicon sequencing, qPCRs targeting ARGs and downstream bioinformatics data pre-processing to enable analysis dynamics in gut microbiota composition and ARGs in travellers. The microbial diversity (Shannon) of the travellers' gut microbiota trended towards a decline directly after arrival to the index country (Figure 1A). Subsequently, several travellers showed a decreased microbial diversity within 5 days after travel as compared to their last sample collected during to travel (Figure 1A). Despite these apparent drops in diversity after arrival at the travel destination and again upon travel return, generalized linear mixed-effects models showed no statistically significant changes between pre-travel, during travel and post-travel time periods (all Tukey-adjusted p>0·2; Figure 1B;). This suggests that there are no consistent shifts in microbial diversity on a population level. Indeed, considerable inter-individual variability in changes in Shannon diversity was observed (Figure 1C), suggesting host-specific microbiota dynamics in response to exposures within the different time periods.

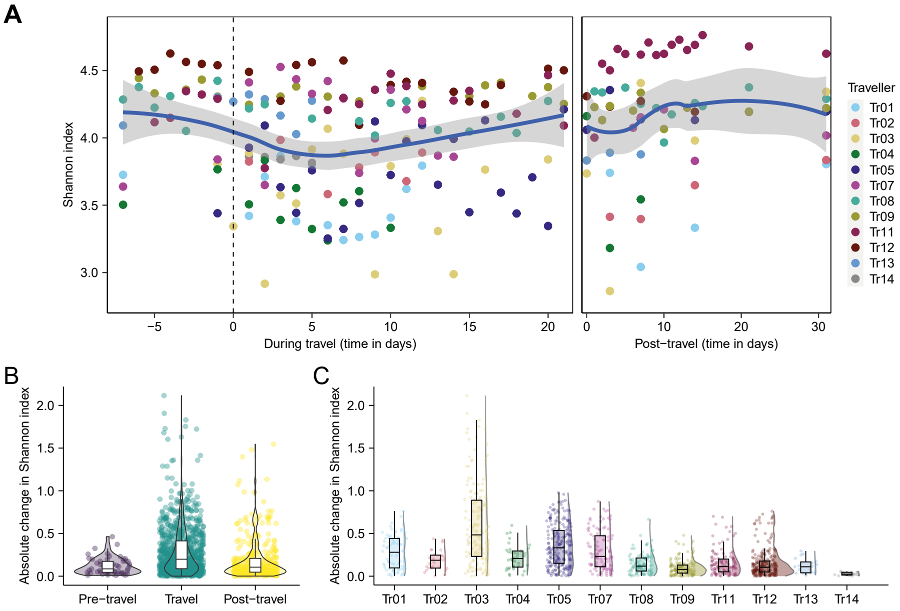

Figure 1. A) Shannon diversity over time per traveller. Blue line depicts fitted Locally Estimated Scatterplot Smoothing (LOESS) curve. The standard error (SE) confidence band is displayed in grey. Dashed line depicts the day of departure. B) Intra-individual absolute changes in Shannon

Figure 1 *Continued*

diversity, showing pairwise comparisons between samples within each individual per period. C) Inter-individual absolute changes in Shannon diversity. Box plots denote the median, IQR, and 95% quantiles.

Travel induces temporary reductions in gut microbiota stability amid individual signatures

Next, we investigated the microbial community composition. Principal Component Analysis (PCA) demonstrated clear inter-individual differences in composition, with each individual maintaining a unique microbiota profile over time (Figure 2A and 2B). Notable exceptions are Tr01 and Tr04 as these observations come from the same person and appear to cluster together in the PCA plot (Figure 2A). Intra-individual variation in microbiota composition differed significantly across time periods, with the highest day-to-day dissimilarity observed during travel, indicating reduced microbial stability compared to pre- and post-travel (Tukey-adjusted p<0·0001; Figure 2C).

Consistent with the inter-individual differences in microbial diversity dynamics, we also observed substantial variation in compositional stability across travellers, with some showing highly stable microbiota and others exhibiting pronounced temporal fluctuations (Figure 2D).

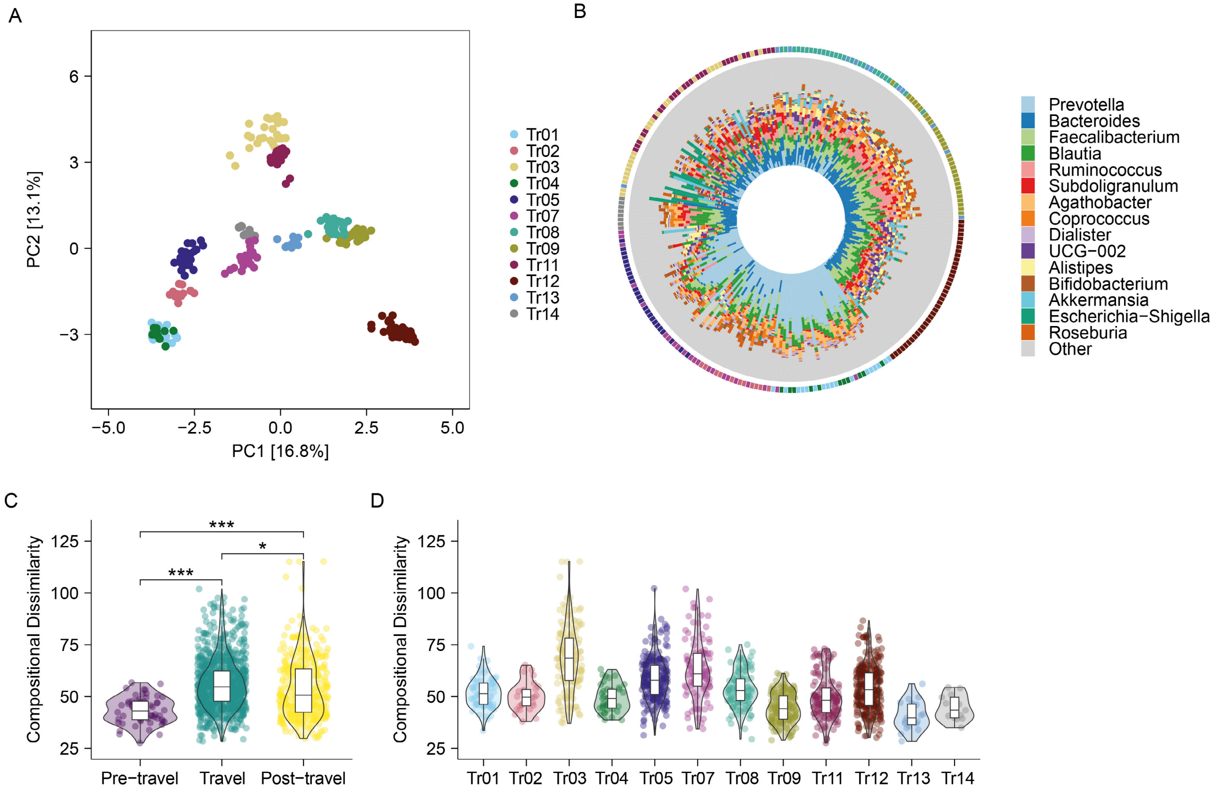

Figure 2. A) PCA visualizing beta-diversity of the traveller cohort during all time periods (prior to travel, during travel and post-travel) combined with a circular bar plot (B) depicting on the outer layer the traveller. C) Intra-individual compositional dissimilarity per period, shown as pairwise Aitchison distances between samples within each individual per period (linear mixed effects modelling and post-hoc estimated marginal means analysis and corrected for multiple testing with Tukey's HSD, *** = P <0·001, * = P <0·05). D) Inter-individual compositional relatedness of travellers, based on pairwise Aitchison distances. Box plots denote the median, IQR, and 95% quantiles.

To further explore the differences in microbial community structure between each time period (before-, during- and after travel), we visualized the dynamics in microbial (Shannon) diversity and composition for each of the travellers separately (Figure 3, Supplementary Figs 1-10). Day to day variation in Shannon diversity and bacterial community structure was observed with differences between individuals but also between time periods within the same individuals.

As an example, distinct clustering of samples per period was observed in the PCA of Tr12 (Figure 3A). The Euclidean distance between subsequent samples of this female traveller prior to travel was lower compared to the distances observed between subsequent samples within the other time periods (during travel and post-travel), indicating a more stable microbiota profile prior to travel. Large fluctuations of Shannon diversity and relative abundance of several genera were observed during travel as characterized by increases and decreases of *Bacteroides*, *Akkermansia* and members of the *Ruminococcaceae* family. These shifts in microbial diversity and community structure emphasize the reduced stability of the gut microbiota during travel.

In another example, the male traveller Tr11, who accompanied Tr12 to Malaysia, had distinct clustering of pre-travel and travel samples while the post-travel samples clustering partly overlapped with the others and showed highest temporal instability (Figure 3B). Here, the shift of gut microbiota was characterized by fluctuations in *Subdoligranulum* and *Clostridia UCG-014*. Although Tr11 suffered from diarrheal episodes on day 14 and day 15 of travel, no large reductions in Shannon diversity were observed except for the initial drop during the first day of arrival. Noticeably, the offspring (Tr08 and Tr09, both females) of Tr11 and Tr12 also displayed different clustering between pre-travel, during travel and post-travel samples in their individual PCA's (Supplementary Figs. 7, 8). Their samples clustered in between samples of Tr11 and Tr12 (Figure 2A), suggesting a partially shared microbiota between the children and their parents. Given that this family engaged in a round-trip backpacking journey and consumed local street food for most of the trip, representing a marked departure from their usual diet, findings suggest that abrupt dietary shifts may contribute to the pronounced microbiota changes observed.

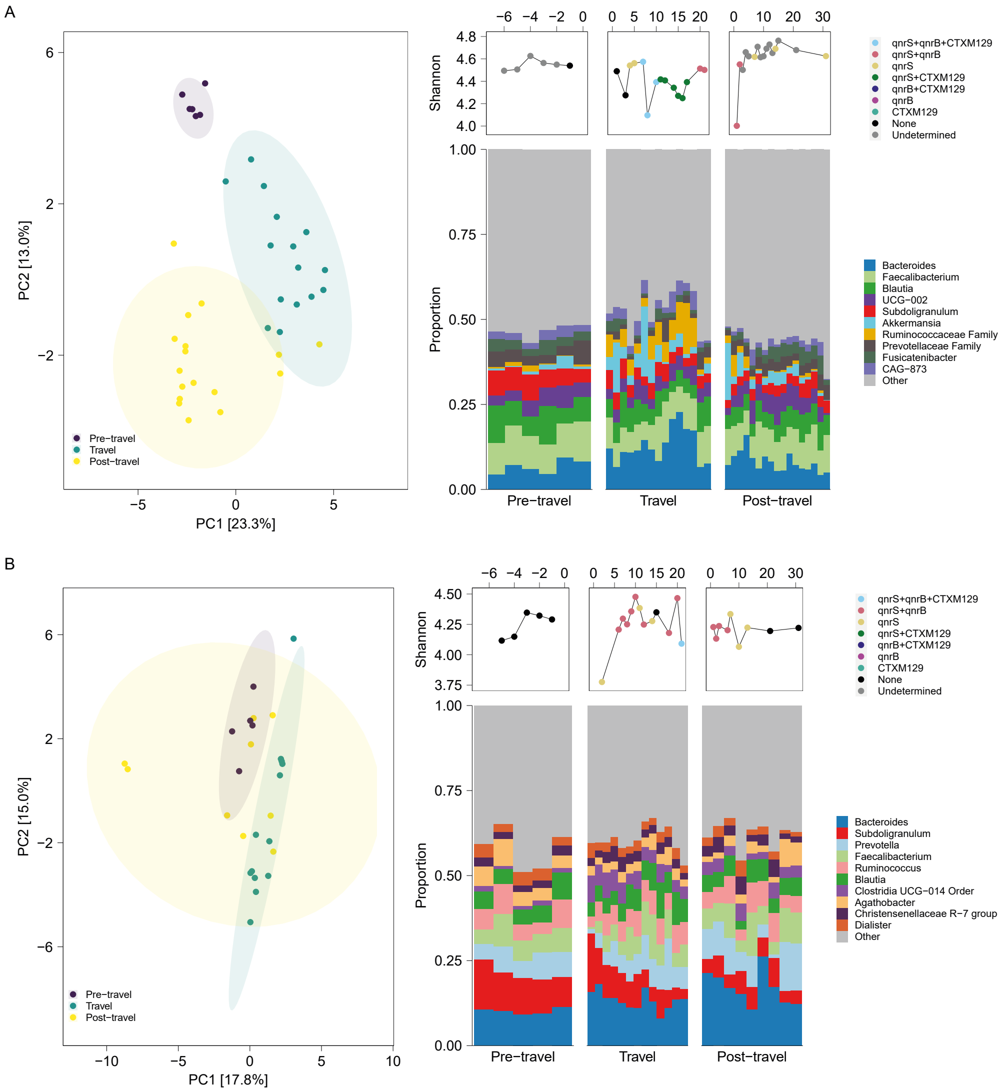

Figure 3. (A) Microbial composition and diversity in traveller subjects over time of Tr12 (top plots) and (B) Tr11 (bottom plots). Principal component analysis (PCA) of the gut microbiota coloured by time period (prior to travel, during travel and post-travel). Shannon diversity is plotted over time, with collection points coloured by the presence of antimicrobial resistance genes (ARGs) detected at each time point. Bar plots show the relative abundance of the top 10 bacterial genera per time period with bars positioned chronologically by sampling time points within each period.

Rapid and frequent acquisition of ARGs in travellers

Thereafter, we performed qPCRs targeting ARGs encoding clinically relevant extended-spectrum beta-lactamases (*bla*$_{\text{CTX-M}}$ group 1, 2 and 9) and plasmid-mediated quinolone resistance (*qnrS*, *qnrB*). Analyses revealed that all the participants had newly acquired at least one these AMR gene during travel, absent in their pre-travel samples. The most acquired ARGs were associated with quinolone resistance. The *qnrB* gene was detected in faecal samples of 8 of the 10 travellers who did not carry this gene prior to travel. Notably, intestinal acquisition of *qnrB* already occurred as soon as at the 2nd day of travel for one participant (Tr04, India). The persistence of *qnrB* carriage varied widely between participants. For one other traveller (Tr01, South-Korea), *qnrB* was detected at only a single time-point during travel, whereas for Tr09 *qnrB* remained detectable until the final sample collection at 3 months post-travel return. For Tr03, hand skin swabs were found to be positive for *qnrB* on days 2, 3 (Supplementary Fig. 11), after which this gene was also recovered from the participant's faecal swabs from travel day 6 onwards, suggesting faecal-oral transmission.

The *qnrS* gene, absent in all pre-travel faecal samples, was acquired by all travellers. In 3 out of 8 travellers with follow-up samples at one month post travel return *qnrS* could still be detected. Acquisition of *bla*$_{\text{CTX-M}}$ genes was detected in 7 participants (Figure 4A), with its earliest acquisition detected on the first day of the trip to Tr13 (India). Hand skin swab collected during Tr04's trip was positive for *bla*$_{\text{CTX-M}}$ on days 2 and 6 (Supplementary Fig. 11), while the faecal samples collected during that trip remained negative.

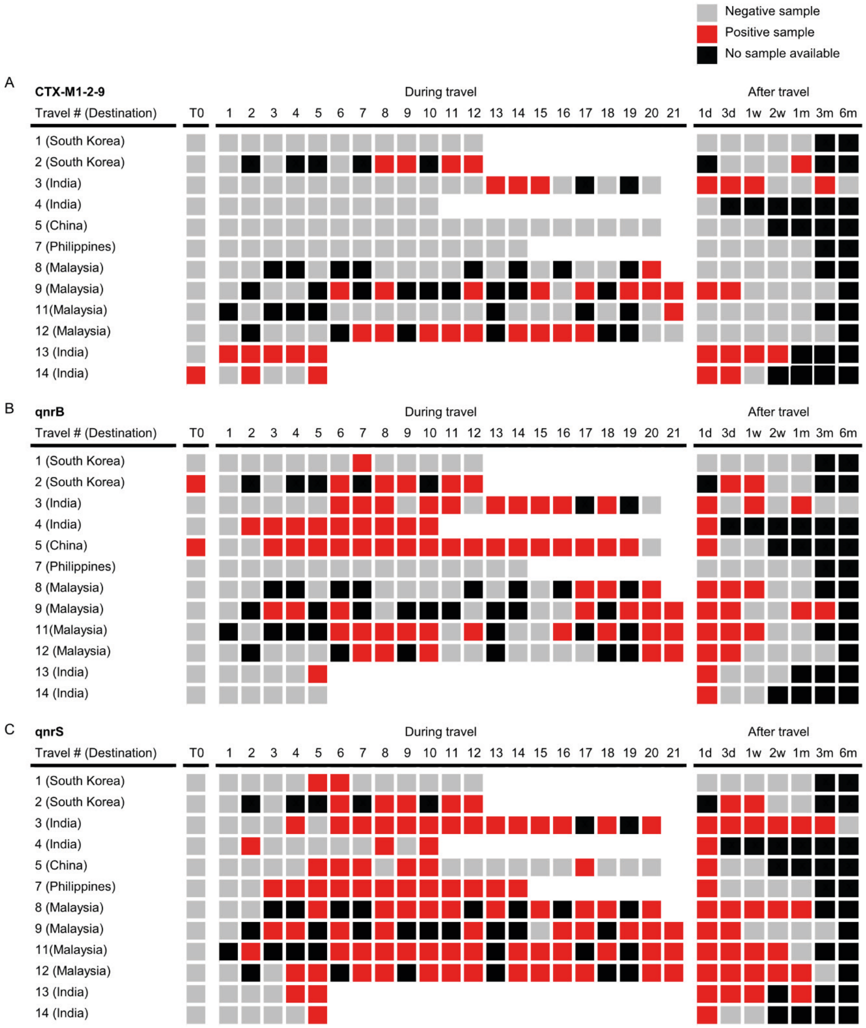

Figure 4. Presence of (A) bla$_{\text{CTX-M}}$, (B) qnrB and qnrS (C) genes in faecal samples before, during or after international travel. Grey squares represent samples which were negative, while red squares represent those which were positive. Black squares represent time points at which no sample was collected. T0: before travel; 1d: 1 day; 3d: 3 days; 1w: 1 week; 2w: 2 weeks; 1m: 1 month; 3m; 3 months; 6m: 6 months.

Enterobacterales expansion and compositional shifts in commensal taxa accompany ARG acquisition during travel

We next investigated whether the gut microbiota was associated with the acquisition or presence of AMR genes. To examine whether a lower microbial diversity increased the risk of ARG acquisition during travel, we limited our analysis to *bla*$_{\text{CTX-M}}$ as *qnrB* and *qnrS* were acquired by (almost) all participants. No significant association was found between Shannon index and *bla*$_{\text{CTX-M}}$ acquisition (LMM; p=0·08; Supplementary Fig. 12). Also, when examining the association between the presence of ARGs and microbial diversity on a per-sample basis, no association was found between the Shannon index and the presence of *bla*$_{\text{CTX-M}}$, *qnrS* or *qnrB* (LMM; all p>0·2; Supplementary Figs. 13A, 13B and 13C).

Finally, we evaluated the shifts in the abundance of dominant microbial taxa in the travellers' gut microbiota, focussing on pre-travel and during travel samples. Overall, we observed a statistically significant increase of *Enterobacterales* during travel (LinDA; FDR<0·0001; Figure 5) which coincided with the acquisition of one or more ARGs (*bla*$_{\text{CTX-M}}$, *qnrS* or *qnrB*). Following travel, *Enterobacterales* abundance showed a decreasing trend towards prior to travel levels (LinDA, p=0·026; FDR=0·502, Figure 5). In addition, we observed statistically significant decreases in the abundances of *Oscillospirales*, Clostridia vadinBB60 group, *Lachnospirales* and *Bifidobacteriales* during travel (LinDA; all p<0·05, FDR<0·2; Supplementary Fig. 14). Notably, these decreases occur in parallel with the acquisition of ARGs as well as with a decrease in microbial diversity during the initial 5 days of travel (Figure 1A, Figure 4).

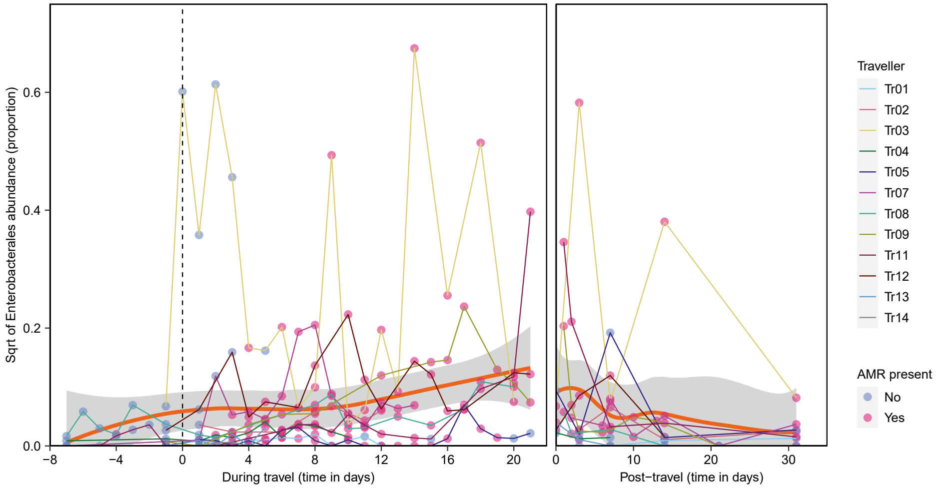

Figure 5. Increase in Enterobacterales abundance over time. Orange line depicts fitted Locally Estimated Scatterplot Smoothing (LOESS) curve. Dots connecting the lines depict the individual samples and are coloured pink when at least one of the studied ARGs was detected and blue when the sample was negative for all ARGs. Sqrt = Square root. The standard error (SE) confidence band is displayed in grey.

Discussion

Here we present the first study employing daily sampling to elucidate the temporal dynamics of gut microbiota composition and AMR gene acquisition during intercontinental travel and showed that compositional shifts and acquisition of AMR genes can occur within days of arrival at the travel destination. While the species diversity remained overall stable across study periods, the results showed an overall initial decline within the first 5 days of travel, which coincided with the earliest detection of AMR genes, as well as overall decreases in several bacterial taxa. This early period appeared to be critical for microbial shifts, suggesting a narrow window during which travellers are most susceptible to acquire resistant organisms.

Previous research has established that travel can influence gut microbiota and/or facilitate the acquisition of AMR genes[13-15,20-23]. However, these studies often relied on pre- and post-travel sampling, potentially overlooking rapid and transient microbial changes observed from our results. By implementing a daily sampling regimen, our study provides a granular view of the gut microbiota's responsiveness to external stimuli during travel. This approach allows for the detection of short-lived microbial fluctuations that might be missed with less frequent sampling intervals.

A previous study by Kantele et al.[24] investigated the acquisition dynamics of ESBL-producing Gram-negative bacteria in 20 European travellers visiting Laos and highlighted the transient nature of colonization and the rapid acquisition of multiple resistant strains during travel. This aligns with our own findings of swift AMR genes acquisition, the increase of *Enterobacterales* during travel as well as the transient and sometimes brief nature of AMR gene persistence.

In addition, the initial decline of commensal bacteria combined with the presence of AMR genes during the early period of travel suggests that an altered colonization resistance due to microbiota perturbations caused by travel is potentially involved in AMR acquisition risk. Future research involving this critical early window is necessary to uncover the role of microbial perturbations in resistome dynamics.

Interestingly, in every participant included in this study, at least one of the targeted antibiotic resistance genes (ARG) was detected, indicating that ARG acquisition during travel to regions in Asia prevalent with resistant bacteria is common and even short visits to high-risk environments pose a substantial risk for AMR gene uptake. While precise prevalence rates cannot be inferred due to the limited cohort size, these findings are consistent with earlier studies[23,25,26]. The *qnrS* gene has been reported at high frequencies in bacterial isolates and faecal metagenomes from several Asian countries, reinforcing the notion that regional AMR burden directly influences ARG acquisition in travellers[24,25].

Moreover, genes from the $\textit{bla}_{\text{CTX-M}}$ family were detected in travellers to India, South Korea, and Malaysia. These genes are known to be widespread in Asia, and previous studies have shown that a significant proportion of travellers returning from South and Southeast Asia carry multidrug-resistant Enterobacteriaceae, often harbouring $\textit{bla}_{\text{CTX-M}}$ genes[25,26]. Notably, we detected $\textit{bla}_{\text{CTX-M}}$ in hand skin swabs of one of travellers to India, while faecal sample of the same individual remained negative. This suggests that although colonization of the gut microbiota may not always occur or remains undetected, there can still be significant exposure to ARGs through environmental contact. This emphasizes the importance of hand hygiene as a preventive measure.

Our cohort of only 11 participants may be considered a limitation due to not being able to fully capture the variability of gut microbiota across diverse populations. In addition, some practical challenges such as variations in individual bowel habits or low biological material during faecal swabbing may have led to missing samples on certain days as well as occasional failures in AMR gene detection due to low DNA yield respectively. The latter could explain the presence of intermittent negative samples interspersed among ARG-positive samples. This potentially leads to an underestimation of how quickly and frequently ARGs are acquired and highlights the need for careful and consistent sample collection methods in future studies.

In conclusion, the daily sampling methodology employed in this study offers a novel and detailed perspective on the gut microbiota's dynamics during international travel. The identification of the initial five days post-arrival as a critical period for microbial shifts and AMR gene acquisition highlights the need for timely preventive measures. Future research should build upon this approach to further explore the factors influencing early microbiota changes and AMR acquisition during travel and to develop targeted interventions aimed at reducing the risk of AMR dissemination associated with global travel.

Methods

Study population and sample collection

In this prospective cohort study, a total of 11 healthy Dutch volunteers who were planning to travel outside of Europe and had not used antibiotics in the previous 3 months were recruited at the Maastricht University Medical Centre. The participants were asked to collect faecal swabs before, during and after an international trip to Asia. Faecal swabs were collected multiple times during the week prior to travel, on each day that defecation occurred during travel, and again at 1 and 3 days, as well as at 1, 2 and 4 weeks after returning from travel. For some travellers, additional samples were collected at 3 and/or 6 months post-travel return.

To explore whether carriage of AMR bacteria on the skin preceded intestinal colonization, we additionally collected swabs from the palm and fingers of the dominant hand at any given point during the day, preferably consecutively during the first 3-7 days of travel in a subgroup of six participants. All swabs were immediately stored in DNA/RNA Shield (Zymo Research, Irvine, USA) to ensure the stability of the metagenome during unrefrigerated transport.

Moreover, participants also provided information on travel dates and destinations, gastrointestinal infections as well as antibiotic use during the study period.

Ethics declaration

All participants provided written informed consent. The study was in accordance with the Helsinki Declaration and was assessed by the Maastricht University Medical Center medical ethics committee. The medical ethics committee has declared that this study does not fall under the Medical Research Involving Human Subjects Act (METC 2018-0701).

Sample processing and DNA extraction

The faecal and hand swabs (DNA/RNA Shield) were stored at ambient temperature during the participants' travels. DNA/RNA Shield stabilizes DNA and RNA at ambient temperature, inactivates pathogens, and is validated for diverse sample types such as blood, faeces, and swabs. They were subsequently stored at -20°C in the lab until DNA extraction. Identical sampling methods were employed for all subjects. The extraction of DNA was performed as described previously[27]. Briefly, 200 µl of DNA/RNA Shield, in which the swabs were suspended, was added to a 2-mL vial containing 0·5 g of 0·1 mm zirconia/silica beads (BioSpec, Bartlesville, OK, USA), 4 glass beads (3.0-3.5 mm)(BioSpec) and 1.2 ml of lysis buffer from the PSP Spin Stool Kit (Stratec Molecular, Berlin, Germany). Samples were disrupted in a MagNA Lyser device (Roche, Basel, Switzerland) in three cycles of 1 min. at a speed of 5500 rpm. Subsequently, DNA was isolated from the samples with the PSP Spin Stool Kit, according to the manufacturer's instructions. In addition to the samples, negative sampling and four DNA isolation controls as well as two ZymoBIOMICS® Microbial Community Standard (D6300; Zymo Research, Irvine, CA, USA) mocks as positive control were included during DNA isolation and downstream sample handling.

16S rRNA gene sequencing and processing

Amplification of hypervariable V4 region from the 16S rRNA gene was performed using primer pairs 515F (forward: 5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (reverse: 5'-(GGACTACHVGGGTWTCTAAT-3')[27,28]. Following amplification, PCR products from triplicate reactions were combined to reduce variability.

Subsequently, purification was performed using the AMPure XP magnetic bead system (Beckman Coulter, Massachusetts, USA) and eluted in 25 µl of 1× low TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Quantification of amplicons were determined using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, New York, USA) with a Victor3 Multilabel Counter (Perkin Elmer, Waltham, USA). Equal amounts of amplicons from each sample were pooled to ensure uniform representation in the sequencing library. Paired-end sequencing was performed on an Illumina MiSeq platform (Illumina, San Diego, California, USA), utilizing the MiSeq Reagent Kit v3 (2 × 250 cycles) and incorporating 10% PhiX.

The sequencing data was pre-processed, using an in-house pipeline based upon DADA2 consisting of reads filtering, identification of sequencing errors, dereplication, inference and removal of chimeric sequences. Further details are available in Appendix 1 (Supplementary methods). Data were expressed as Amplicon Sequence Variants (ASVs). Samples with a total read count of <10,000 were excluded from further analysis.

Real time PCR assays

Real-time PCR was performed to detect the following ARGs: *qnrB, qnrS* and *bla*$_{\text{CTX-M,}}$ as described previously[29]. These genes were selected because their abundance was significantly elevated after travel[29].

The *qnrB, qnrS* and *bla*$_{\text{CTX-M}}$ were amplified on an Applied Biosystems QuantStudio 5 Real-Time PCR System using TaqPath™ qPCR Master Mix (Applied Biosystems) with cycling conditions according to the manufacturer. The probes to detect *bla*$_{\text{CTX-M}}$ groups 1 and 2 were combined in a single multiplex reaction, while a separate qPCR reaction was used to detect *bla*$_{\text{CTX-M}}$ group 9.

Statistical analysis

The study utilized a convenience sample; therefore, power calculations were not performed. All downstream analysis was performed in R (v.4.1.3). Alpha and beta diversity was calculated using R packages 'phyloseq (v1.38)' and 'microviz (v0.10.1)' while plots were constructed in 'microviz' and 'ggplot2 (v3.4.3)'. To minimize the impact of spurious low-abundance features, ASVs representing less than 0·01% of the total reads across all samples were excluded from downstream analyses.

To account for scale invariance and the compositional characteristics of sequencing-derived count data, we estimated the relative abundance profiles by modelling the data using a Dirichlet distribution, implemented with 128 Monte Carlo simulations via the ALDEx2 package (v1.24.0). The resulting values were subjected to a centred log-ratio (clr) transformation to mitigate the constraints inherent in compositional microbiome data[30].

Pairwise dissimilarity between samples was quantified using the Aitchison distance, which represents the Euclidean distance between clr-transformed vectors, and was computed using the vegan package (v2.6-4). This distance metric served as the basis for evaluating beta diversity. Sample ordination was performed through principal component analysis (PCA) on the Aitchison distance matrix. The ordination plots were further integrated with metadata using the microViz package, and final visualizations were produced with ggplot2.

To model the effect of travel on gut microbial diversity, generalized linear mixed-effects models (GLMMs) with a gamma distribution and log link were fitted by maximum likelihood using the glmmTMB package (v1.1.7). To assess the effect of travel on gut compositional dissimilarity, linear mixed-effects models (LMMs) were fitted by maximum likelihood using the lmer function in R. Models were specified to evaluate changes in α-diversity and β-diversity, with time period (pre-travel, during travel, post-travel) included as a fixed effect and traveller included as a random effect. In addition, LMMs were used to assess the association between *bla*$_{\text{CTX-M}}$ acquisition and Shannon diversity, and between the presence of *qnrB, qnrS* or *bla*$_{\text{CTX-M}}$ and Shannon diversity. Post hoc pairwise comparison between different time periods (e.g., Pre-travel vs during travel) was performed on fitted models (for α-diversity and β-diversity) using emmeans package (v1.10.5), with Tukey's Honest Significant Difference (HSD) adjustment for multiple testing. Significance was set at $P<0.05$ (Tukey-adjusted).

Differential abundance analysis was performed using Linear Models for Differential Abundance (LinDA, R package v0.1.0) to identify microbial taxa associated with travel and post-travel. Taxonomic count table at the Order level was used as the response matrix. Orders present in less than 60% of the samples were excluded to reduce the influence of sparsely represented taxa and enhance the robustness of the analysis (prev.cut = 0.6). In the model formula, time was included as continuous variable while also adjusting for country travelled, age, sex, as well as inter-individual variability by specifying subjects as the random effects. P-values were corrected for multiple hypotheses using Benjamini–Hochberg method (FDR), and significance was set at FDR < 0.2.

Data Availability

Metagenomic sequence data will be made available publicly with publication at the European Nucleotide Archive under Bioproject PRJEB96287

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Author contributions

PW and JP designed the study. CvW was responsible for sample handling and microbiology. CvW, PW and JP were involved in data curation. CvW, JC, and JP accessed and verified the data. JC conducted the statistical analysis. PS, NvB, PW and JP did supervision and consultation. JC and CvW wrote the first draft, which was edited and reviewed by all authors. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

The authors declare no competing interests.

Supplementary data

Supplementary tables are available online at the following repository: https://surfdrive.surf.nl/s/TzLJsfgaYJKqKdn

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Appendix

Daily sampling reveals rapid microbiota alterations and antimicrobial resistance gene acquisition during intercontinental travel

Supplementary methods

Pre-processing sequencing data. The final length of the forward and reverse reads were 240 and 160 nucleotides, respectively. The standard filtering setting was used with the enforcement of maximum of 2 expected errors per-read. Subsequently, the reads were dereplicated to remove identical sequences and inferenced and merged to obtain the full denoised sequences. Sequences with forward and reverse reads overlapping by at least 12 bases, and those that were identical to each other in the overlap regions were aligned together. To assign taxonomy, DADA2 [1] was used to annotate up to the genus level using the database SILVA 138 version 2 [2]. Decontam [3] was applied for the identification and removal of contaminant ASVs using the “either” option, which integrates both the prevalence and frequency statistical approaches to detect contamination in marker-gene and metagenomic datasets.

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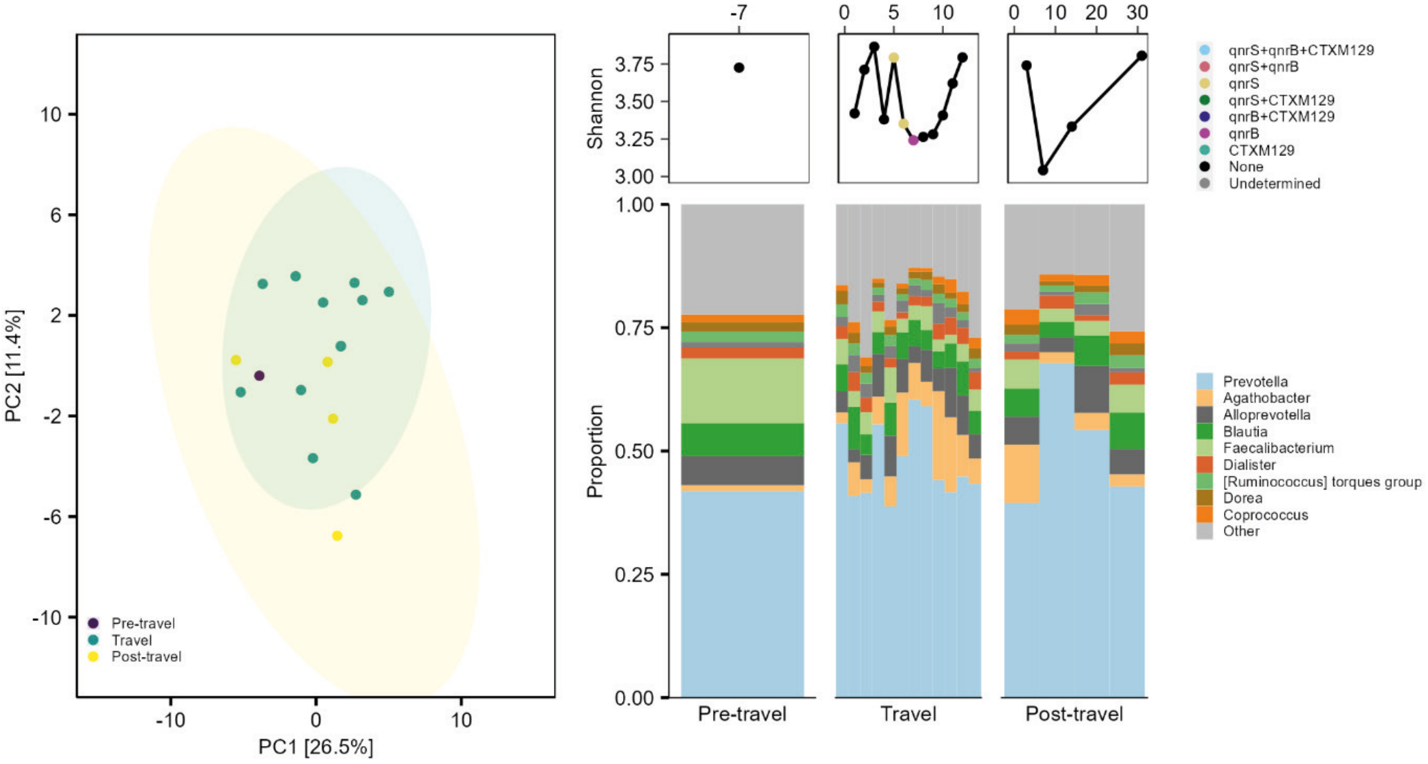
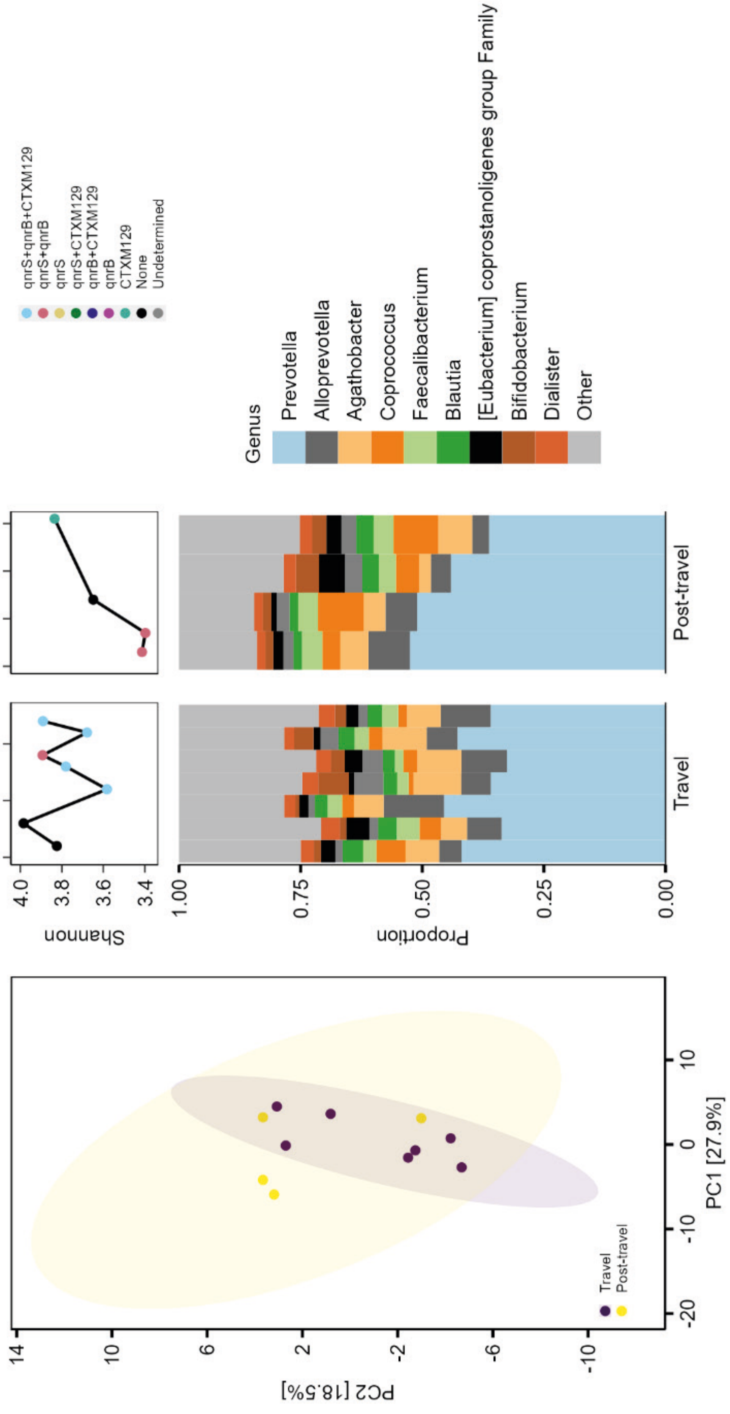
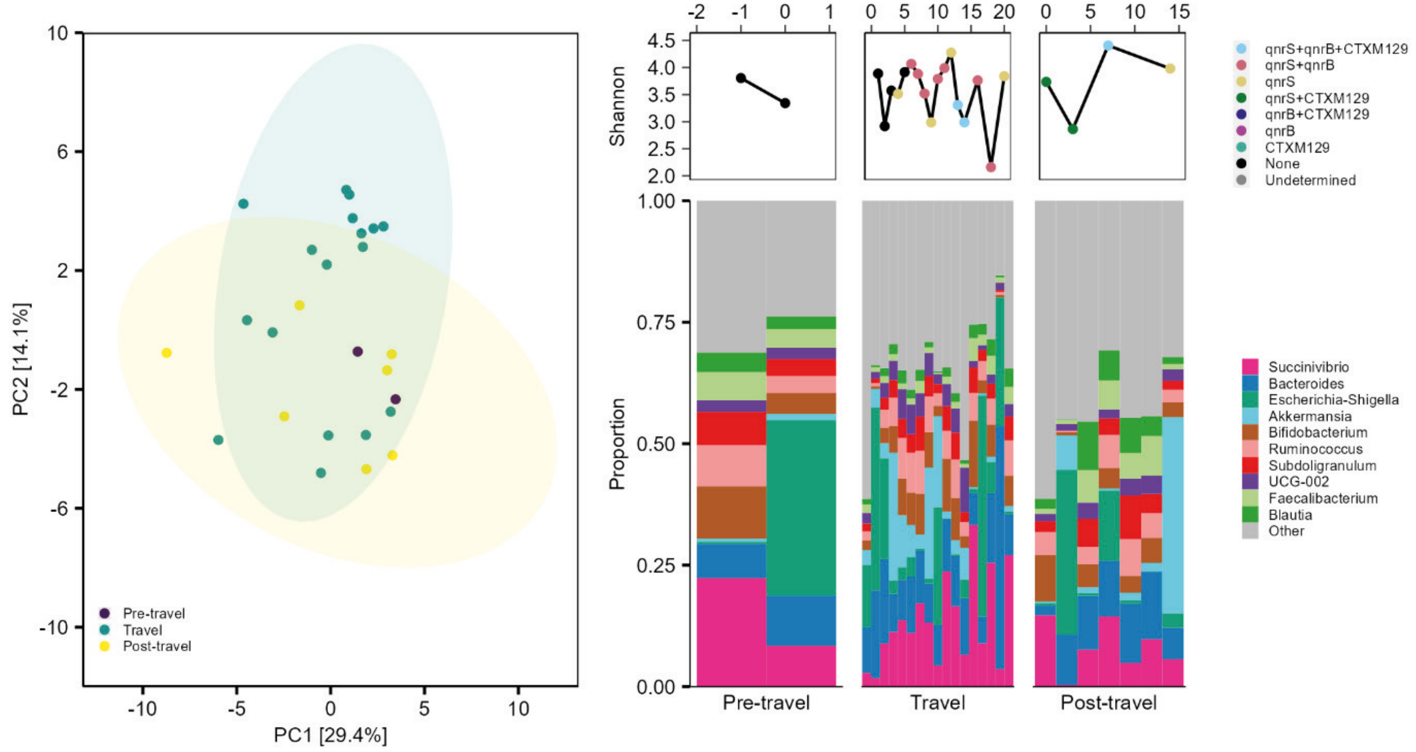
Supplementary Figs. 1-10. Microbial composition and diversity profiles of traveller subjects over time. Microbial composition and diversity in traveller subjects over time. Principal component analysis (PCA) of the gut microbiota coloured by time period (prior to travel, during travel and post-travel). Shannon diversity is plotted over time, with collection points coloured by the presence of antimicrobial resistance genes (ARGs) detected at each time point. Bar plots show the relative abundance of the top 10 bacterial genera per time period with bars positioned chronologically by sampling time points within each period.



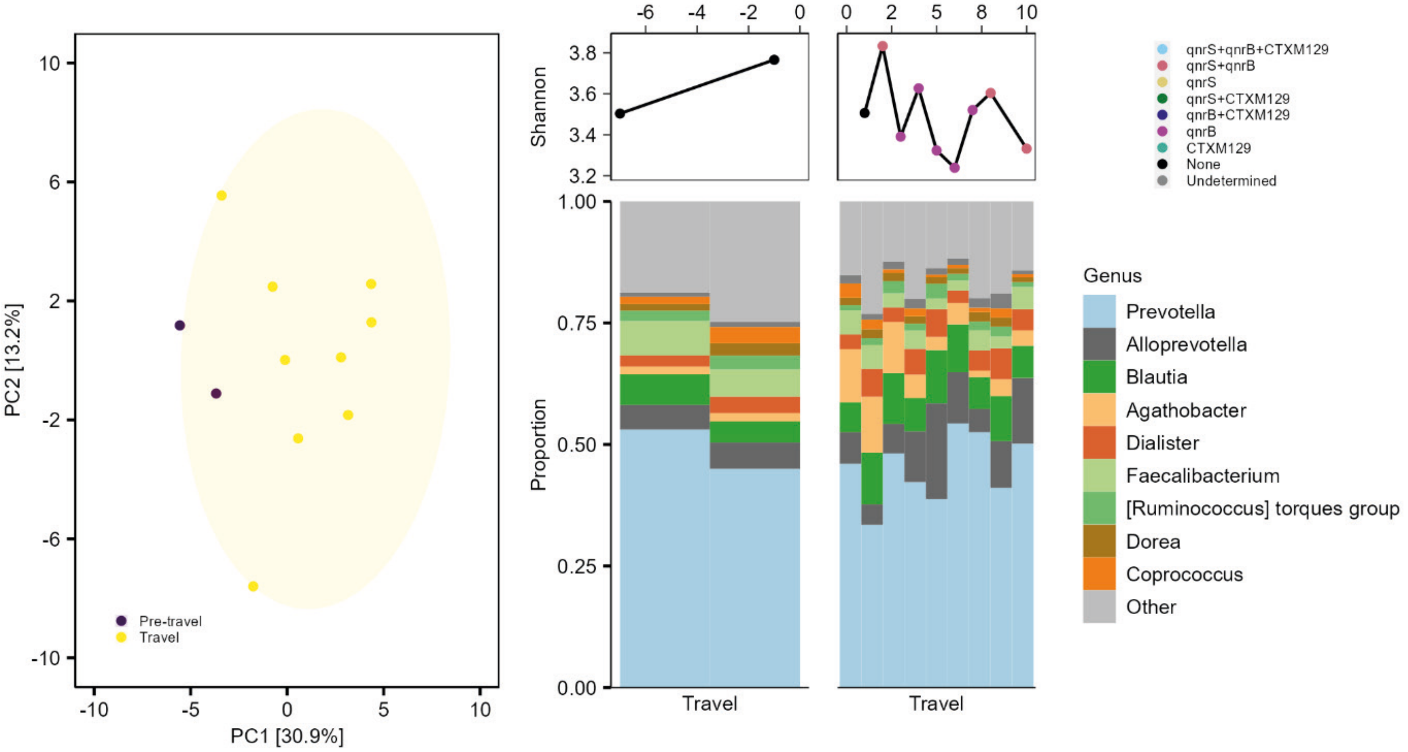
Supplementary Figure 1. Traveller Tr01



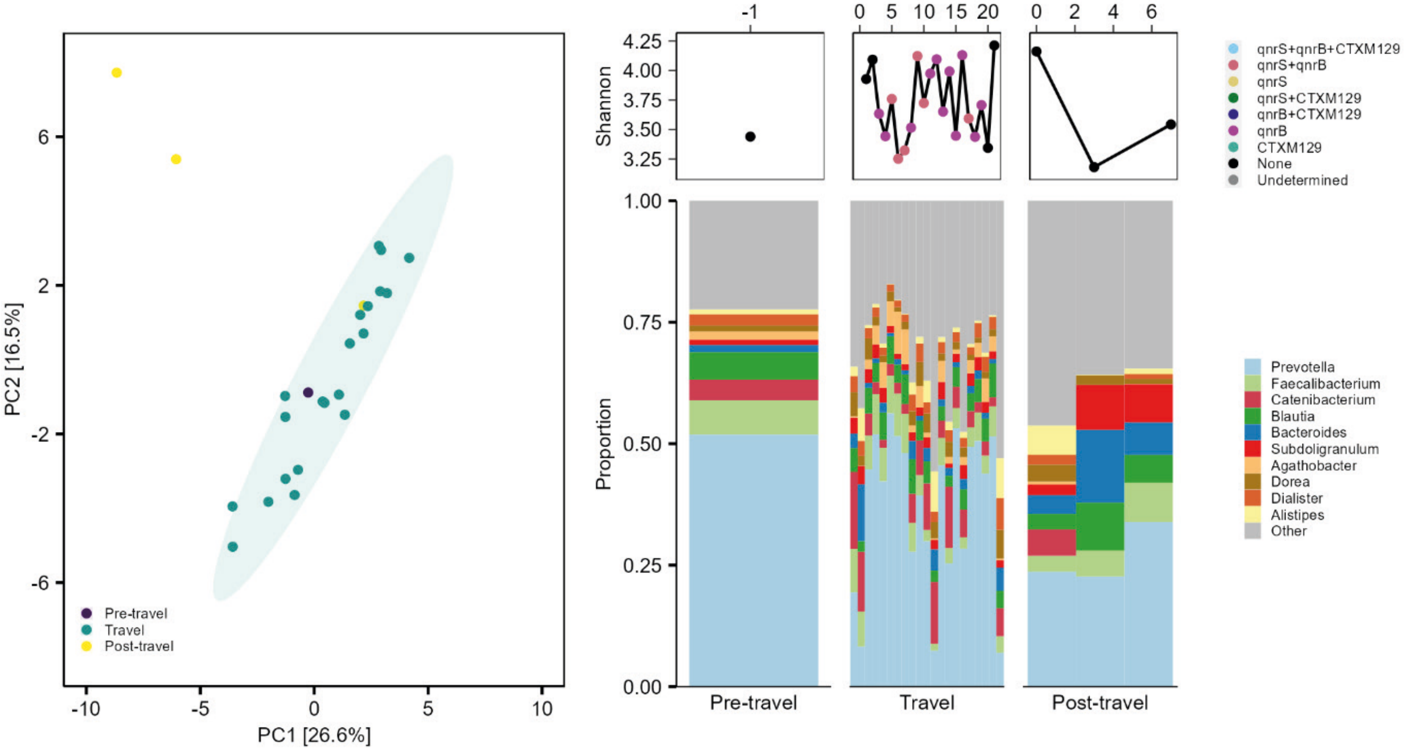
Supplementary Figure 2. Traveller Tr02



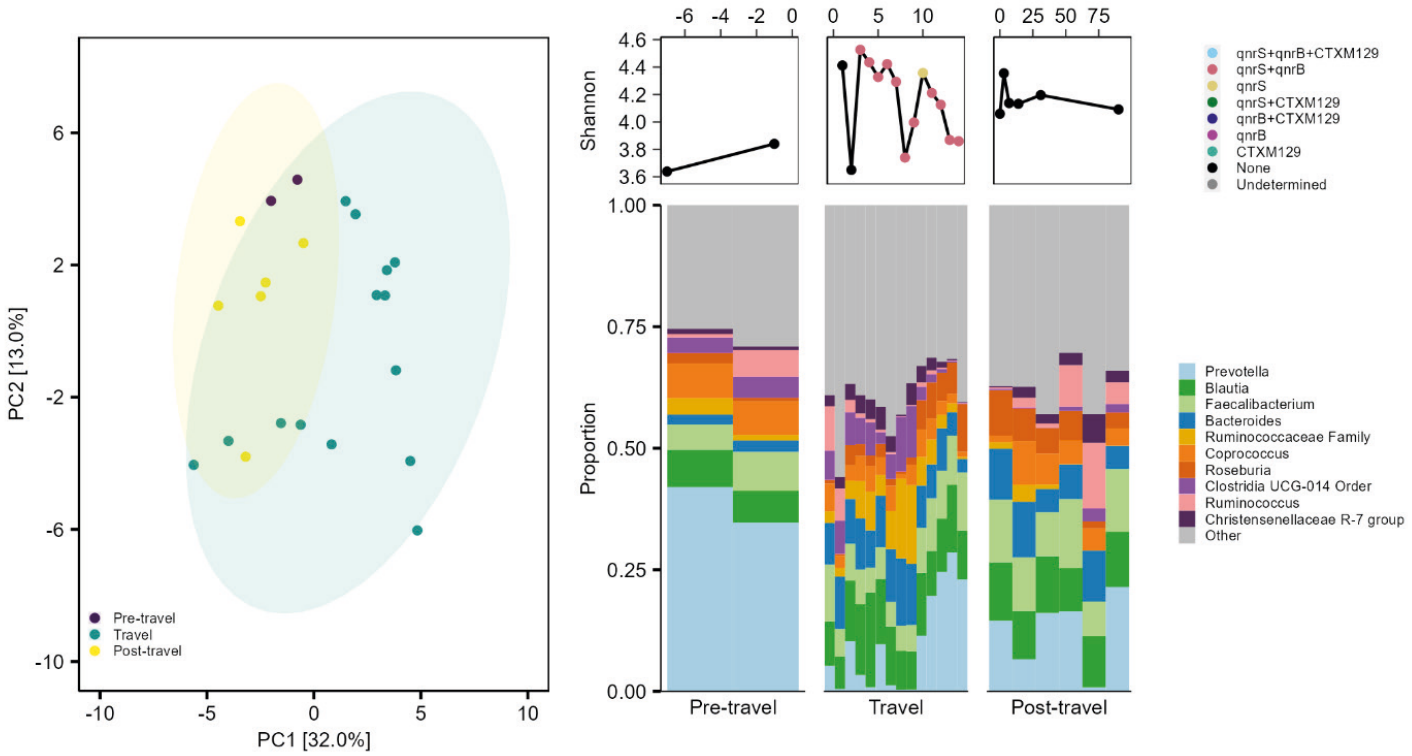
Supplementary Figure 3. Traveller Tr03



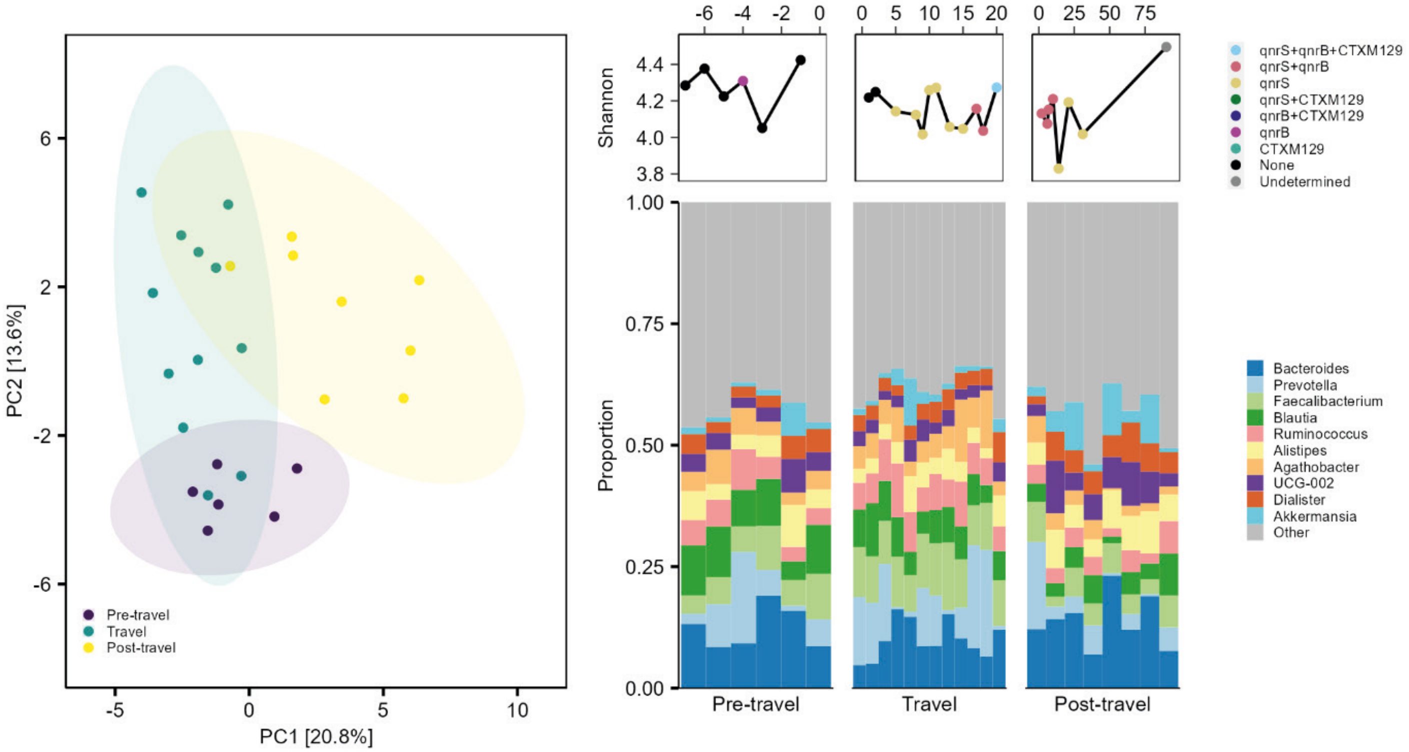
Supplementary Figure 4. Traveller Tr04



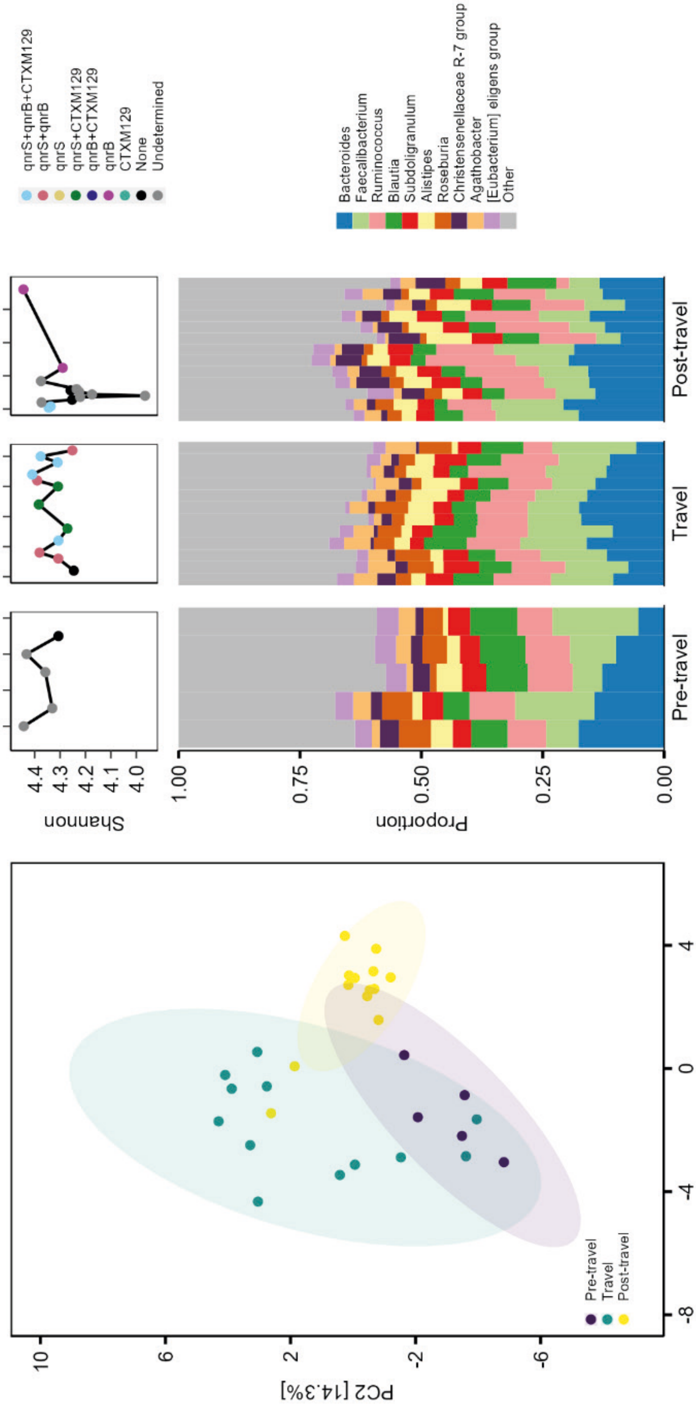
Supplementary Figure 5. Traveller Tr05



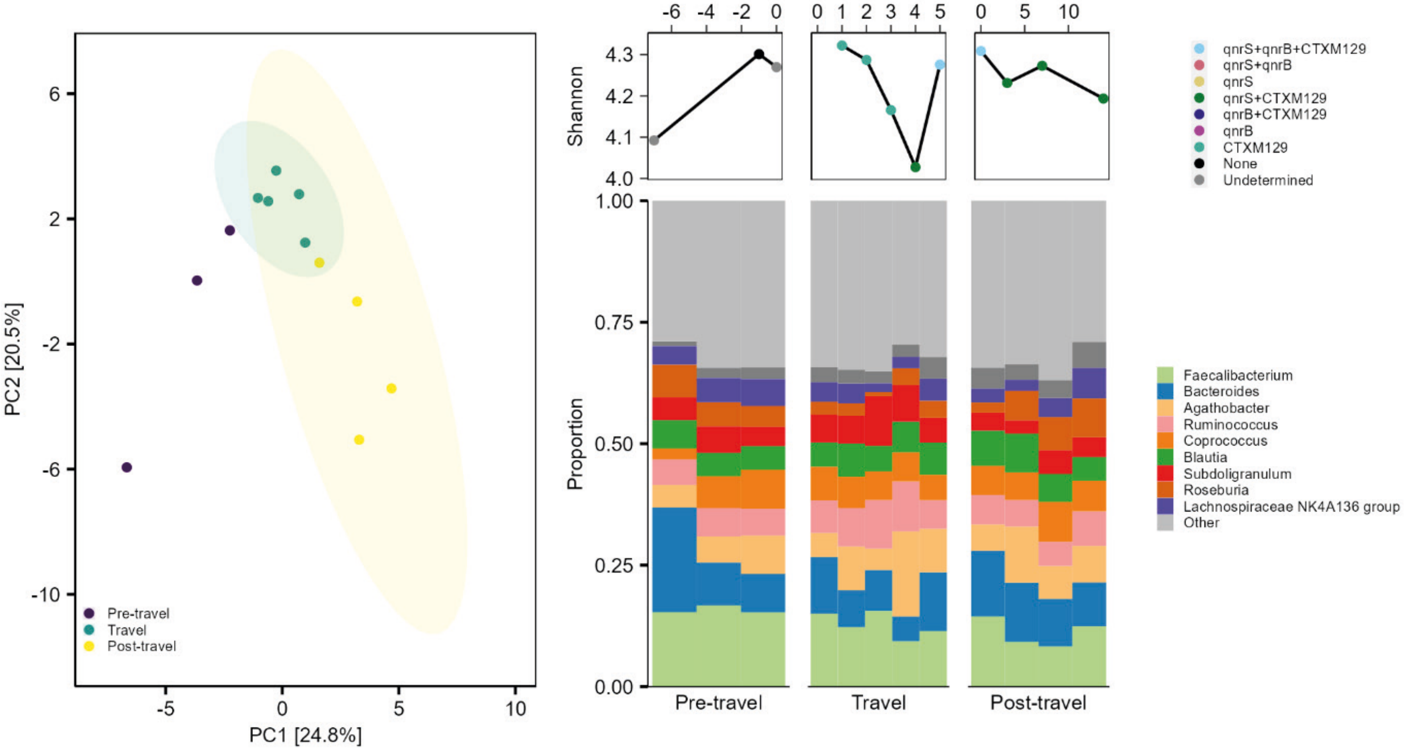
Supplementary Figure 6. Traveller Tr07



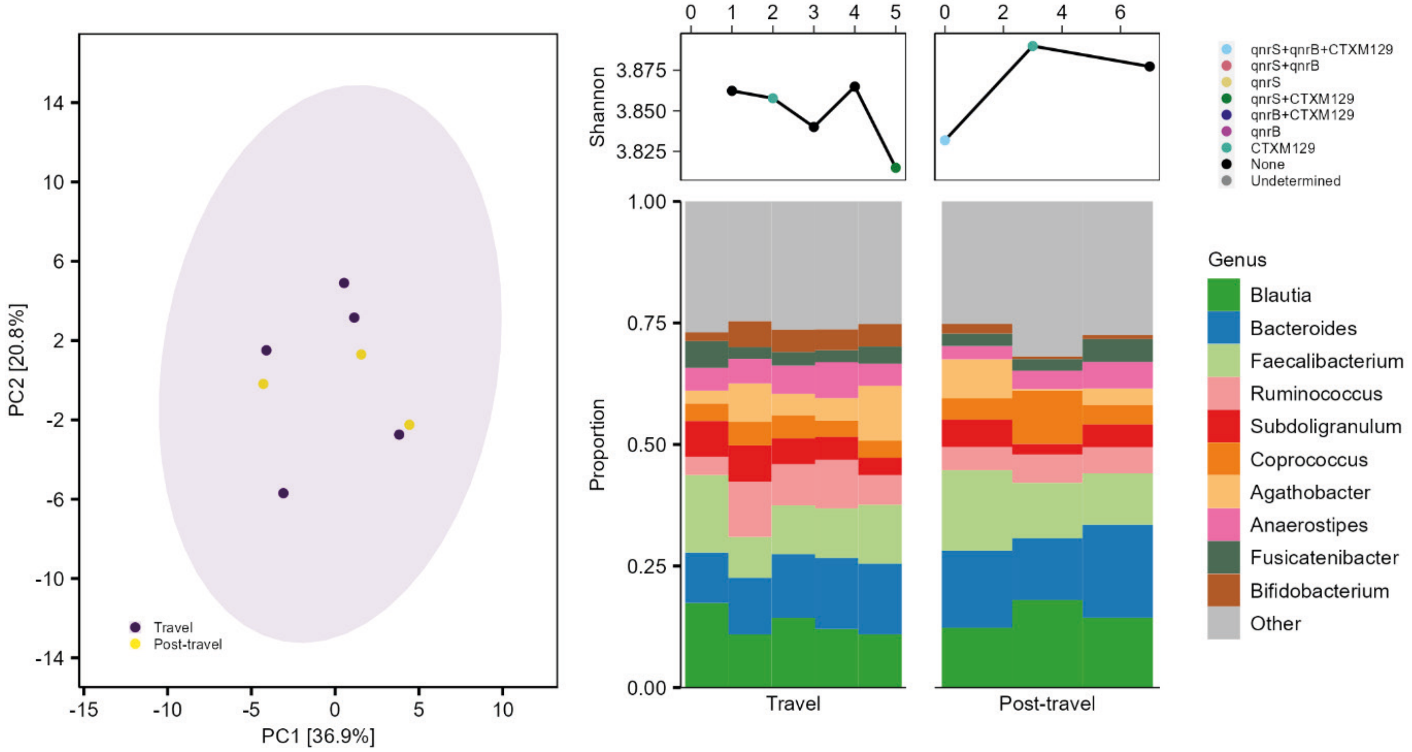
Supplementary Figure 7. Traveller Tr08



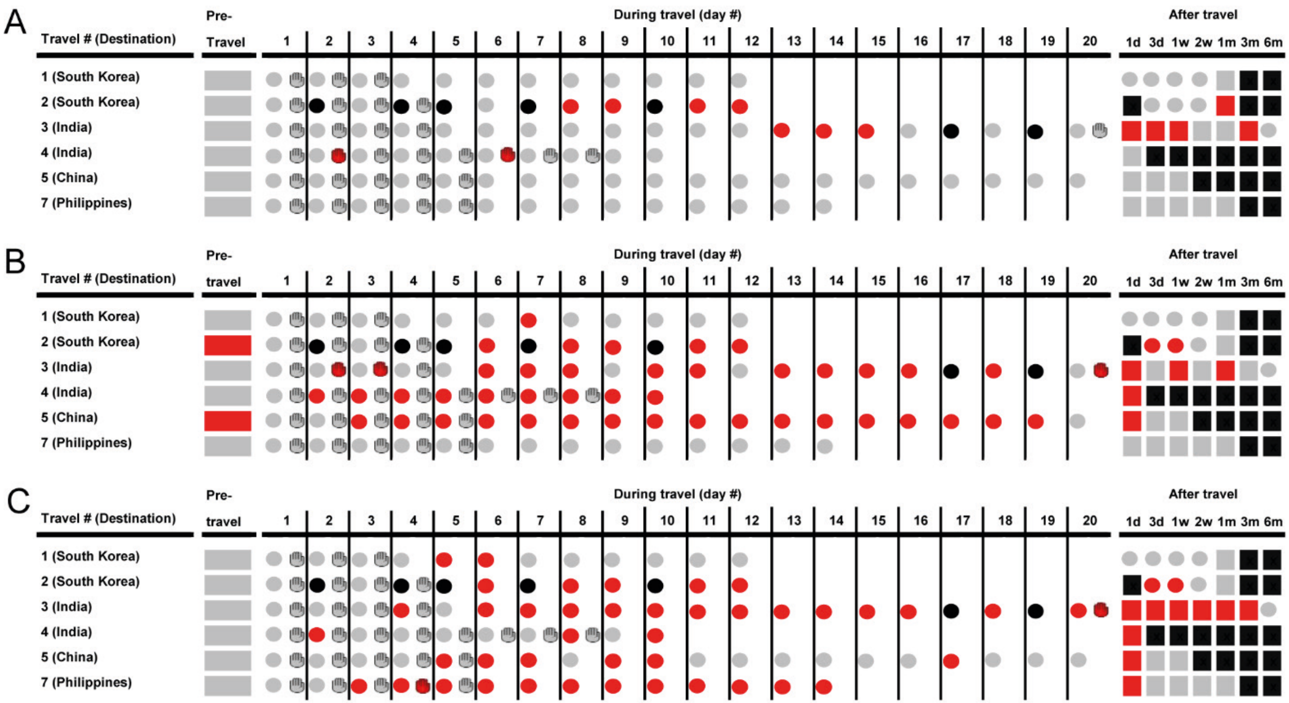
Supplementary Figure 8. Traveller Tr09



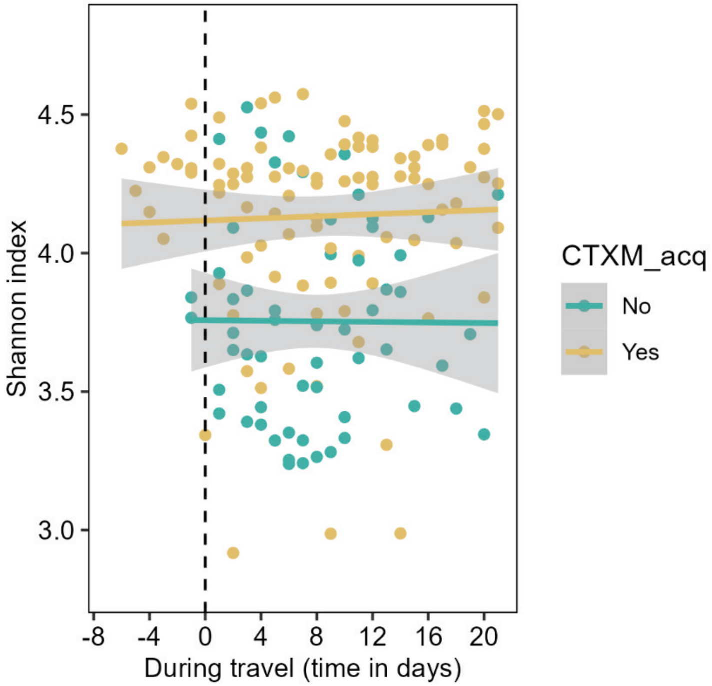
Supplementary Figure 9. Traveller Tr13



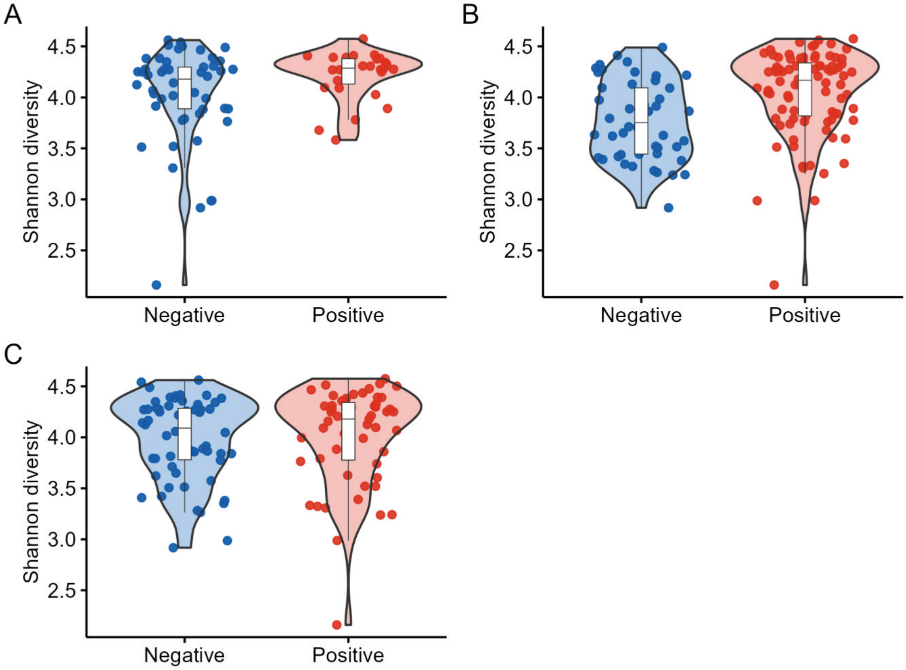
Supplementary Figure 10. Traveller Tr14



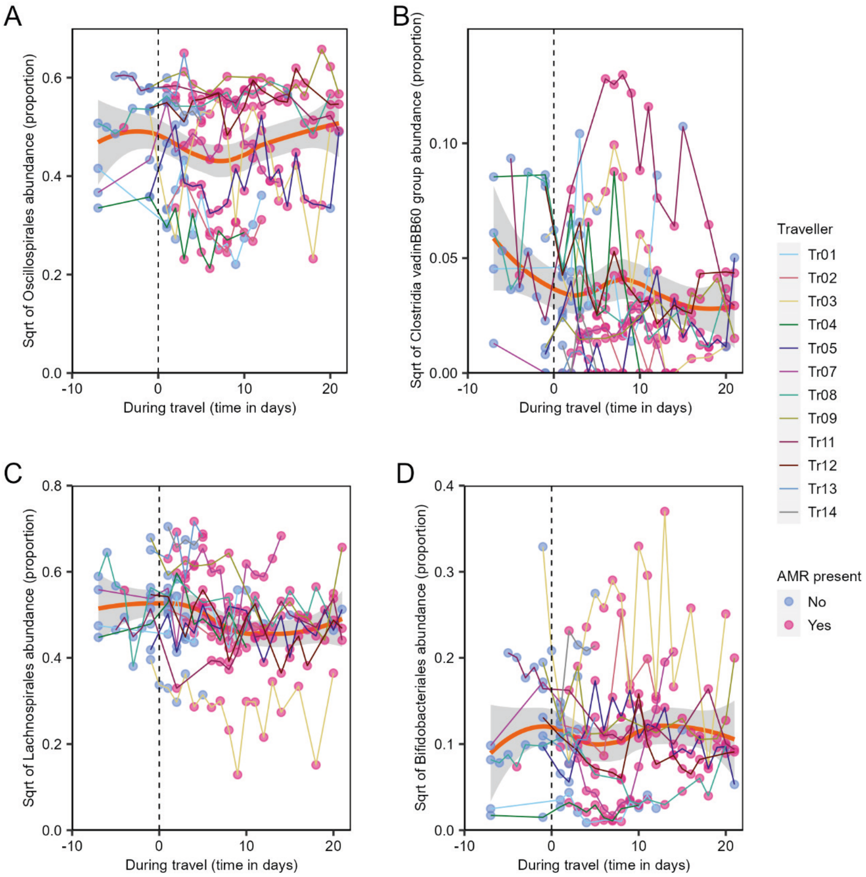
Supplementary Fig. 11 Presence of (A) $bla_{CTX\text{-}M}$, (B) qnrB and (C) qnrS genes in faecal or hand skin swab metagenomes before, during or after international travel. Square, circle and hand symbols represent faecal samples, faecal swabs, and hand skin swabs respectively. Grey symbols represent samples which were negative, while red symbols represent those which were positive. Black symbols represent time points at which a faecal sample or swab was not collected. Only hand skin swabs which were collected are shown in the figure. 1d: 1 day; 3d: 3 days; 1w: 1 week; 2w: 2 weeks; 1m: 1 month; 3m; 3 months; 6m: 6 months.



Supplementary Fig. 12 Comparison between samples of travellers that did or did not acquire bla$_{\text{CTX-M}}$ during their travels. Dots represent individual samples and are coloured yellow for all samples of travellers that acquired bla$_{\text{CTX-M}}$ at any given time-point during travel or green for all samples of travellers that remained negative for bla$_{\text{CTX-M}}$. The standard error (SE) confidence band is displayed in grey.



Supplementary Fig. 13 Comparison between Shannon diversity of samples with positive (red) or negative (blue) outcome for ARGs. (A) bla$_{\text{CTX-M}}$, (B) qnrS and (C) qnrB. Box plots denote the median, IQR, and 95% quantiles.



Supplementary Fig. 14 Relative abundance profiles of Oscillospirales (A), Clostridia vadinBB60 group (B), Lachnospirales (C) and Bifidobacteriales (D) over time, from pre-travel (day -7 to day 0) till during travel (day 1 to day 21). Orange line depicts fitted Locally Estimated Scatterplot Smoothing (LOESS) curve. Dots connecting the lines depict the individual samples and are coloured pink when at least one of the studied ARGs was detected and blue when the sample was negative for all ARGs. Sqrt = Square root. The standard error (SE) confidence band is displayed in grey.

CHAPTER 7

General Discussion

As these chronicles of the travelling gut conclude, it is worth remembering that intercontinental travel is often perceived as a cultural and social experience, yet from a biological perspective it represents a large-scale natural experiment on human–microbe interactions. As outlined in **Chapter 2**, each journey exposes travellers to new microbial communities, dietary shifts, and environmental stressors that can disrupt or reshape the delicate balance between host and microbiota. In this context, human travel offers a unique opportunity to investigate how the gut microbiota, the body's largest microbial ecosystem, responds to abrupt and potentially lasting ecological transitions. This thesis addresses this by integrating epidemiological, microbiological, and metagenomics approaches to characterise the multifaceted effects of intercontinental travel on the gut ecosystem. From an epidemiological perspective, the occurrence of post-infection irritable bowel syndrome (PI-IBS) following traveller's diarrhoea (**Chapter 3**) and the acquisition of antimicrobial resistance genes (ARGs) among travellers (**Chapter 5**) represent distinct, yet interconnected outcomes of travel related microbial and host perturbation. With this thesis, I underscore travel as both a clinical and public health challenge: it can precipitate long-term gastrointestinal morbidity in some individuals, while simultaneously facilitating the dissemination of antimicrobial resistance determinants across borders. Building upon these epidemiological insights, subsequent investigations (**Chapters 4** and **6**) explore the underlying microbial dynamics that accompany and potentially mediate these outcomes.

Gut microbiome dynamics during travel

The studies presented in this thesis demonstrate that the gut microbiota responds to travel with measurable shifts in composition. These dynamics, explored in detail in **Chapters 4** and **6**, reveal that alterations can occur within days of departure and that recovery trajectories vary widely between individuals. In addition, interpretation is complicated because the gut microbiome is shaped by many external and internal factors, including medication use, circadian rhythm, infections, and diet as reviewed in **Chapter 2.** A practical response is to use large longitudinal cohort designs with rich covariate capture, allowing change to be judged against an individual baseline or conceptual healthy reference. Such approach was implemented in this thesis as **Chapter 4** revealed how the gut microbiota in travellers was moderately different directly after travel compared to pre-travel.

In **Chapter 6** we showed how quickly the microbiota can respond to environmental changes. Day-to-day fluctuations in composition occur even in healthy individuals, and specific taxa may expand or contract transiently without lasting consequences[1-3]. We observed transient increases in Enterobacterales during travel, an order that includes both commensals and opportunistic pathogens such as *Klebsiella*, *Salmonella*, and *Escherichia coli*. Other studies have shown that fluctuations in *Enterobacteriaceae*, a family in the order Enterobacterales, occur as part of a “normal” microbiome response to environmental exposures[1,3]. Interestingly, such early blooms have also been observed during short-term antibiotic exposure in healthy adults, followed by recovery toward baseline[4]. In contrast, recovery of Enterobacterales was only evident once individuals returned to their home environment, suggesting that travel introduces more complex or sustained perturbations involving ongoing environmental exposure, colonization by exogenous strains, or dietary changes. Restoration of the native environmental context may be a prerequisite for re-establishing the pre-travel microbial equilibrium. Therefore, investigating such patterns can offer a framework for understanding how communities recover after disturbance, as recovery after perturbation is oftentimes a hallmark of a healthy microbiome. While the microbiome is generally resilient, resilience has limits, and persistent dysbiosis may arise when recovery is incomplete or altered[5]. For example, antibiotic exposure can result in the permanent loss of specific taxa[4], and dietary changes can remodel community structure[1,6]. As such, persistent change in Enterobacterales (and Enterobacteriaceae) may reflect a state of perturbation and dysbiosis.

Still, increases in Enterobacteriaceae have also been observed after acute infections, which represent a qualitatively different form of perturbation. This more severe perturbation can trigger inflammation thereby elevating electron acceptors like nitrate and oxygen which in turns confers a fitness advantage to Enterobacteriaceae and thus driving blooms during infection possibly leading to persistent dysbiosis[7]. Consequently, it is mechanistically not just the presence of a single pathogen but the interaction with the broader microbial consortium that determines whether recovery is complete or dysbiosis persists [8-10].

PI-IBS in travellers

Irritable bowel syndrome (IBS) is a chronic disorder involving gut–brain interaction, defined by recurrent abdominal pain with altered bowel habits[11].

Within this spectrum, some individuals develop IBS after an episode of intestinal infection, termed post-infection IBS, which usually involves diarrhoea-predominant or mixed bowel habits and a clearly identifiable onset[12]. Framing PI-IBS as a definable phenotype within IBS supports targeted investigation of its development. In travellers, travellers' diarrhoea (TD) is a common precipitant, with attack rates typically around 20 to 50 percent[13]. Among those who experience TD, a meaningful minority develop PI-IBS. **Chapter 3** estimated an incidence of approximately 12 percent of PI-IBS among subjects experiencing TD, consistent with prior meta-analyses reporting 10 to 15 percent[14,15]. Notably, PI-IBS incidence after TD varies by travel destination, underscoring how risk is shaped by local environments, pathogen exposure, and host-pathogen interactions. **Chapter 3** highlighted higher PI-IBS rates following travel to South Asia compared with Latin America or Africa, likely reflecting regional differences in circulating pathogens and sanitation standards[16,17]. These differences are not only a function of infection risk but also of the types of organisms encountered. Protozoal infections, in particular, are associated with the highest risk of PI-IBS, followed by bacterial gastroenteritis, while viral infections appear to contribute less consistently[18]. In addition, both prolonged and clinically severe diarrhoeal episodes are strong predictors of PI-IBS, supporting the idea that the magnitude of an acute insult shapes the likelihood of chronic sequelae[15,18]. In **Chapter 3**, we observed that nearly 80% (48/61) of travellers who developed PI-IBS still met diagnostic criteria one year after travel which underscores the persistence of this condition. This challenges the prevailing view of TD as a short-lived illness and instead frames it as a potential initiating event with long-term consequences for gastrointestinal health and quality of life. In addition to infectious and clinical risk factors, psychosocial and demographic influences remain highly relevant. **Chapter 3** confirms that female sex, pre-existing anxiety, and psychological stress are consistently associated with increased risk of PI-IBS, suggesting that vulnerability arises at the interface of infection, host physiology, and psychosocial environment[18]. Despite strong epidemiologic evidence for PI-IBS, mechanistic explanations are still lacking. Previous data on microbial signatures in PI-IBS broadly suggest that the gut microbiome may provide an important key to the underlying pathophysiology[18-20].

In **Chapter 4** we investigated the role of the gut microbiota in PI-IBS development and found that the microbial profile differed even before travel, thus prior to symptom onset. Travellers who later developed PI-IBS showed lower microbial diversity pre travel and experienced a more pronounced decline in diversity after diarrhoeal illness compared with those who did not develop symptoms.

Together, these observations suggest that individuals who later develop PI-IBS may already carry a gut microbial community that is less resilient to perturbation. A microbiome with lower baseline diversity is generally thought to have reduced functional redundancy and diminished ecological buffering capacity, which may make it more vulnerable to the destabilising effects of an acute infectious insult such as traveller's diarrhoea. In this framework, the infection acts not as the primary cause of dysbiosis but as a trigger that exposes an underlying susceptibility: a less diverse ecosystem is more easily pushed into an altered or maladaptive state from which recovery is incomplete. This interpretation provides a conceptual bridge between our pre-travel findings and existing mechanistic literature on diversity, colonisation resistance, and microbiome resilience, and helps to explain why only a subset of exposed individuals progress to PI-IBS despite experiencing a similar infectious event. Previous longitudinal studies in non-travel settings showed that the adult gut microbiome is generally stable, with diversity resilient to many short-term perturbations such as dietary shifts, though more substantial exposures, including antibiotic therapy, consistently reduce diversity[3,21,22]. Lower diversity is often associated with decreased colonisation resistance and increased vulnerability to persistent disturbance, particularly following antibiotic use[4]. Recent experimental work in gnotobiotic mice further supports a diversity–protection link: as community diversity increases, a greater fraction of nutrients is competitively consumed, limiting pathogen growth and enhancing colonization resistance[23]. However, diversity alone is an imperfect marker of health and stability as gut transit time and stool moisture also influence diversity. For example, constipation has been linked to increased diversity, whereas diarrhoea is associated with lower diversity[3,24]. Thus, while diversity metrics provide a useful overview of community structure, they cannot capture the specific taxa driving ecological change.

Indeed, taxa profiling in **Chapter 4** revealed differences between new-onset PI-IBS cases and controls, with several taxa consistently enriched or depleted across pre-travel, travel, and post-travel sampling. Notably, *Eggerthella lenta*, repeatedly associated with anxiety, IBS and PI-IBS[25-29], carries strain-specific immunomodulatory and metabolic activities that can influence Th17 signalling and host compound metabolism[30-33]. Other enriched taxa, including *Bacteroides fragilis* and *Ruminococcus gnavus*, were also previously implicated in IBS and possess functions linked to mucosal and immune disruption, including mucin degradation, neuromodulatory metabolite production, and pro-inflammatory signalling[34-37]. In contrast, the identities of the species or genera depleted in new-onset PI-IBS compared with healthy controls remain poorly resolved, underscoring the need for further work to determine whether these taxa, including understudied Clostridia groups, may exert beneficial or protective effects in PI-IBS development.

Furthermore, gastrointestinal infections remain as pivotal initiating events precipitating post-infection conditions such as PI-IBS[38]. In **Chapter 4**, we showed that EAEC virulence genes were among the most frequently detected directly after travel and displayed the greatest persistence at one-month post-travel in PI-IBS cases, based on quantitative Polymerase Chain Reaction (qPCR) analysis. EAEC is also one of the most common pathogens causing TD[16,39]. Although EAEC infections generally result in less severe infections than pathogens like *Campylobacter jejuni*, they are well known to results in a longer duration of symptoms (around 3–14 days)[39] and may therefore increase the risk of PI-IBS. Previous meta-analyses show that the risk of PI-IBS varies by pathogen, with parasites carrying the highest risk, followed by bacteria and then viruses[14,15]. However, **Chapter 4** did not include comprehensive coverage of pathogens beyond diarrhoeagenic *E. coli*, nor did it provide sufficient resolution to stratify PI-IBS risk according to specific pathogen yet. Nonetheless, causative agents of TD are still difficult to determine as many remain unknown or mixed in a substantial proportion of TD cases. Indeed, a systematic review on TD between 2005–2015 in military and long-term travellers, showed no pathogen could be detected in 36% of diagnostic tests[16]. Switching from conventional assays to qPCR improves diagnostic sensitivity[39,40], yet qPCR also has important limitations. Its high sensitivity may yield false positives by detecting transient low abundant colonization rather than true infection, and it remains restricted to predefined panels of known pathogens. This is also reflected in **Chapter 4**, where we detected certain virulence genes in participants even before travel, consistent with earlier reports showing that EAEC and EPEC virulence genes can be present prior to travel[41,42]. In addition, even multiplexed qPCR fails to identify an etiologic agent in approximately half of cases, as observed in the study by Boolchandani et al., who investigated microbiome and resistome development in TD[43]. In a Finnish cohort of international travellers, even when broad bacterial, viral, and parasitic panels were applied, 28.1% (41/146) of diarrhoea cases remained without a detected pathogen[41].

Such undetected cases may reflect gaps in virulence gene coverage within the panel, missed detection due to intermittent shedding or sampling during the recovery phase, or diarrhoea of non-infectious origin. This highlights the complexity of attributing post-travel gastrointestinal symptoms to a single pathogenic agent and underscores that pathogen detection alone provides only a partial view of the processes underlying PI-IBS. As shown in **Chapters 3 and 4**, and supported by previous research, the likelihood of developing PI-IBS is further shaped by individual characteristics, behaviours, exposures, and the gut microbiota[14,15,18]. These considerations point to the necessity of moving towards a framework that captures the wider microbial and behavioural contexts to elucidate the pathophysiology of PI-IBS.

AMR in travellers

Unlike the development of post-infection sequelae such as PI-IBS, the acquisition and carriage of ARGs in the gut microbiome typically occurs silently and without clinical manifestation. Travellers are often unaware that their intestinal microbiota has incorporated ARGs, as carriage generally does not present with gastrointestinal symptoms or illness. The exception arises during episodes of TD, where ARG acquisition is more frequently observed[43,44]. Nevertheless, the "silent" acquisition is more concerning as demonstrated in **Chapter 6** where day-to-day monitoring of 11 travellers revealed rapid acquisition and persistent carriage of ARGs. Prior studies have demonstrated that international travel is one of the strongest risk factors for acquisition of resistant Enterobacterales, especially extended-spectrum β-lactamase (ESBL)-producing strains[38,44,45]. The large multicentre COMBAT study, in which the studies described in **Chapters 3**, **4**, and **5** were conducted, previously showed that 34% of Dutch travellers acquired ESBL-producing Enterobacteriaceae during a single trip. Highest acquisition rates were reported among travellers visiting South Asia (75%). This was further substantiated by our extended analysis on the presence of specific ARGs encoding for beta-lactamase, quinolone resistance and mobilized colistin resistance (mcr) as presented in **Chapter 5**. Travel to South Asia was a consistent independent predictor of ARG acquisition across all gene groups, in agreement with other reports showing the rise of AMR in South Asia as well as it being among the highest-burden regions in deaths associated with and attributable to bacterial AMR[46,47]. Destination shaping AMR acquisition mirrors the regional differences in the background prevalence of AMR bacteria driven by differences in sanitation infrastructure, antibiotic consumption, and antimicrobial stewardship. We further established in **Chapter 5** that post-travel ARG prevalence was significantly higher than pre-travel, confirming that travel leads to a measurable expansion of the gut AMR content. Dietary habits such as shellfish consumption also emerged as a risk factor, suggesting foodborne exposure routes. These findings align with environmental studies reporting ARGs, including *bla*$_{\text{CTX-M}}$ and *mcr*, in aquatic food chains and seafood products from Asian markets[48-50]**.** In addition, while **Chapter 5** showed low amounts of people acquiring *mcr* genes, other reports have documented acquisition of these plasmid-mediated colistin resistance genes despite its lower frequency than beta lactam and quinolone resistance[51-54]. Such introductions are particularly concerning, as they further limit therapeutic options for severe infections.

Furthermore, having a chronic disease, frequent food stall consumption, unpeeled fruit intake, antacid use, and female sex were also associated with higher odds of specific ARG acquisition (**Chapter 5**). Underlying chronic conditions and use of antacid drugs may predispose travellers to colonisation by antibiotic resistant bacteria. Meta-analysis found inflammatory bowel disease to double the odds of MDR-E acquisition during travel, indicating that underlying gut pathology increases susceptibility to colonisation by resistant strains[55]. Frequent consumption of street food and unpeeled fruit likely reflects greater exposure to contaminated food, and ingestion of such food is seen as a major route of AMR acquisition during travel[56,57]. Female sex may contribute through differences in health related behaviours and higher rates of antibiotic treated infections such as urinary tract infections[58], although sex stratified AMR susceptibility data remain limited. Together, these findings underscore the interplay between host factors, travel behaviour, and susceptibility to acquiring antimicrobial resistance.

In **Chapter 6**, we further investigated the short-term dynamics of microbiota and ARGs shifts during travel in a high-frequency longitudinal design. The use of daily stool sampling provided an unusually high temporal resolution of microbiota and ARGs dynamics. Most studies capture only pre- and post-travel snapshots [52,53], and consequently miss the timing of critical transitions. In eleven travellers to Asia, we observed rapid microbiota perturbations within the first days abroad, marked by an increase in Enterobacterales and a transient loss of commensal diversity. These microbial changes coincided with the swift occurrence of ARGs in the faecal microbiome. ARG acquisition can occur within days of arrival in high-prevalence regions, and gut microbial instability during travel may facilitate such (temporal) acquisition. This is in line with a previous daily sampling study showing rapid and highly dynamic acquisition of multidrug resistant Enterobacterales during travel[59], and metagenomic studies where the gut resistome expands during and/or immediately post travel in parallel with marked microbiota perturbation[43,52,54]. ARGs acquired during travel in our cohort (**Chapter 6**) declined within three months after return, which mirrors previous work indicating that travel associated MDR colonization is usually transient and that ARG abundance often returns towards baseline within a few months. Interestingly, previous studies reported that a non-trivial subset of around 2 to 10 percent of travellers remain ESBL-E colonised up to 12 months upon travel return[38,60,61]. Other studies have also shown that some clinically important AMR genes, including *mcr-1* or bla_{NDM}, can persist well over 6 months upon acquisition in some individuals [53,62]. Such persistent carriers may act as reservoirs for onward transmission to household contacts and sustain community carriage, contributing to the silent spread of AMR across borders[38,63,64]. Thus, the acquisition of ARGs during travel and subsequent persistence represents a largely hidden yet significant consequence of global mobility and this underscores the importance of long-term monitoring of travellers who travelled to AMR hot zones.

Together, the studies in **Chapters 5 and 6** provide complementary perspectives: the large cohort study (Chapter 5) identifies key determinants and behavioural risk factors associated with ARG acquisition, while the in-depth longitudinal analysis (Chapter 6) reveals how rapidly these changes can occur and how they relate to microbiota dynamics. Both demonstrate that ARG acquisition during travel is frequent, multifactorial, and largely asymptomatic and emphasize that travel, human behaviour, and environmental exposure are closely interconnected in shaping global AMR.

Methodological strengths

Several methodological aspects of this thesis increase its reliability and extend its contribution to the field of microbiome and travel medicine research.

First, the prospective, multicentre design of the large-scale COMBAT study (**Chapter 3**, **4** and **5**) increased generalisability and reduced the risk of selection bias. By recruiting across multiple travel clinics and following participants prospectively, this thesis provides more robust estimates of incidence and risk factors. Importantly, the multicentre approach enabled inclusion of travellers visiting diverse intercontinental destinations, thereby strengthening external validity, and making findings applicable to a broader traveller population.

Second, frequent sampling (**Chapter 6**) allowed the detection of microbial and ARG alterations within days of travel, demonstrating how rapidly these systems respond to environmental exposures. This not only improves our understanding of the kinetics of microbiome perturbation and recovery, but also identified windows of vulnerability that may be targeted for intervention.

Finally, the adoption of complementary analytic approaches, including traditional regression (**Chapter 3** and **5**) and machine learning models (**Chapters 3** and **4**), added methodological rigor to the work. Machine learning helped identify complex, non-linear interactions (e.g. anxiety as a predictor of PI-IBS in **Chapter 3**), while traditional models offered interpretability and robustness. The combination of these methods illustrates how different analytical lenses can generate richer insights than either approach alone. Collectively, these methodological choices support a shift in microbiota and travel medicine research from primarily descriptive associations toward a more dynamic, integrated, and clinically actionable analytical perspective.

Public health and clinical implications

From a public health perspective, international travellers act as vectors for the dissemination of both pathogens and AMR genes. The parallel between symptomatic outcomes, such as PI-IBS (**Chapter 3 and 4**), and asymptomatic carriage of AMR genes (**Chapter 5 and 6**) highlights the dual impact of travel: it poses a direct clinical risk for the individual while simultaneously contributing to broader microbiological and AMR-related transmission dynamics. Both phenomena concentrated among travellers to high-burden destinations such as South Asia, where both environmental exposure to pathogens and baseline levels of AMR are markedly elevated[65,66].

The same acute event that raises PI-IBS risk, in particular prolonged gastroenteritis, is also involved in the increased likelihood of ARG acquisition[18,38,54]. This overlap suggests that severe or prolonged gut disturbances may create windows of heightened vulnerability in which both long-term functional disorders and antimicrobial resistance traits are more likely to arise. It highlights the importance of preventive measures during travel, including careful food and water hygiene and related practices that reduce the risk of extended gastroenteritis. Although pre-travel consultations play a key role in increasing awareness of travellers' diarrhoea risk and other travel-related hazards, such as malaria and hepatitis[67], many intercontinental travellers do not seek pre-travel advice, often because of a low perceived risk[68,69]. In addition, the extensive information provided during such consultations may limit short-term recall[70]. Increasing public awareness of travel-associated health risks, particularly for travel to high-risk destinations, and reinforcing key messages through reminders could improve information retention and encourage greater uptake of pre-travel consultations. However, sustained adherence to preventive measures remains challenging and does not always effectively prevent illness, as exposure risks are strongly influenced by local hygiene and sanitation conditions that are largely beyond travellers' control and depend on national or regional public health policies[71-73].

Nonetheless, our findings further identify several modifiable risk factors that could inform preventive strategies (**Chapter 3 and 5**). In addition to traditional TD prevention advice, there is scope to include guidance on responsible antibiotic use abroad and cautious use of antacids. Our observation in **Chapter 3** that psychological factors, particularly anxiety, contributed to PI-IBS risk resonates with recent evidence emphasising the bidirectional gut–brain axis in bowel disorders[74]. Travel may serve as a trigger that unmasks vulnerability in predisposed individual. Thus, there is value of giving advice to minimize anxiety and stabilize stress responses during travel, including maintaining sleep, hydration, regular meals, and choosing lower intensity itineraries. Management of PI-IBS may therefore require a multidisciplinary approach that integrates gastroenterology, psychology, and dietetics, rather than a symptom-focused strategy alone.

In addition, there is a need for preventive measures that extend beyond individual symptom relief to encompass AMR stewardship and global surveillance. Whole-genome and metagenome-assembled genome (MAG) analyses based on long- and short-read sequencing have shown that AMR genes are particularly concentrated in clinically important taxa, such as *Enterobacteriaceae*, and to a lesser extent in other bacterial groups[75]. Thus, it implies that prioritization of surveillance efforts should be given for these high-risk pathogens/bacteria group. However, only using such a targeted approach may overlook the broader ecological dynamics of resistance. Indeed, several examples exist of antimicrobial resistance genes harboured by gut commensals or environmental microbes that have transferred to opportunistic pathogens, including transfer of *vanA* from enterococci to *S. aureus*[76], *bla*$_{\text{CTX-M}}$ from environmental *Kluyvera* species to *Enterobacteriaceae*[77] and OXA-48-type carbapenemases from waterborne *Shewanella* species to enterobacterial species[78]. Moreover, many gut commensals can act as opportunistic pathogens in different host or environmental contexts and may acquire resistance under selective pressure. Therefore, while focusing on clinically relevant AMR organisms remains essential, monitoring the wider microbiome is equally important, as it represents a vast reservoir for horizontal gene transfer and the evolution of novel resistance mechanisms[79].

The evidence of **this thesis** reinforces international travel as a key driver of microbiome perturbation (**Chapter 4 and 6**) and AMR dissemination (**Chapter 5 and 6**), implying the need for coordinated public health strategies that bridge infection prevention, microbiome recovery, and resistance surveillance across borders. Although intercontinental travel can predispose individuals to developing IBS (**Chapter 3 and 4**) following episodes of gastroenteritis, several other scenarios warrant close monitoring. For instance, a large waterborne outbreak of *Giardia lamblia* in Bergen (Norway 2004), caused by contamination of the city’s drinking water reservoir, resulted in nearly half of the confirmed infected population experiencing persistent gastrointestinal symptoms and post-infection functional disorders years after exposure [80,81]. Another example involves poultry abattoir workers, who are frequently exposed to Campylobacter species from infected chickens [82] are at increased risk of developing IBS symptoms [14]. These findings highlight the importance of considering surveillance and investigation of other populations at risk of gastrointestinal infections and subsequent post-infection sequelae.

Future directions

Towards large cohort designs

Our work as demonstrated in **Chapter 3 to 6** was based on Dutch intercontinental travellers, who may differ in baseline microbiota composition, diet, or health status from travellers originating in other regions. Thus, there is a need for a “broader” research effort by including diverse populations. Large multi-ethnicity cohorts may be necessary to disentangle associations and correlations. In this perspective, a big knowledge gap exists on the impact of

AMR acquisition and loss among travellers from AMR hotzones towards regions with a low prevalence of AMR. Additionally, most travellers in COMBAT[38], VOYAGE-R[61] and other cohorts are travelling for leisure. Particularly, travellers visiting their country of origin to connect with friends and relatives are underrepresented in such cohorts, while they are likely more deeply immersed in the local culture and have different risks for AMR and pathogen acquisition.

Recently, the increase in (metagenomics) data as well as the open access availability of such data have created a growing precedent for combining multiple cohorts or using publicly available data to enhance statistical power for association and causality analyses[83-86]. In addition, archived large sample collections from past projects have gained new relevance with advances in sequencing technologies, enabling retrospective microbiome analyses and providing opportunities to uncover new findings such as cholesterol-metabolizing bacteria from the Framingham study that began in 1948[87]. This creates exciting possibilities for travel studies as well: long-stored stool samples from earlier cohorts can now be revisited with modern metagenomics to track and characterize previously undetectable microbes, and compare past and present travel-associated microbiome dynamics. Such retrospective analyses can reveal how global mobility, antibiotic use, and regional exposures have shaped the microbiome and ARG dissemination over time, offering a powerful complement to contemporary prospective studies.

Towards strain diversity and mechanistic understanding

Gut microbiota analyses in this thesis (**Chapter 4 and Chapter 6**) showed clear travel-associated shifts. We demonstrated that 16S rRNA gene amplicon sequencing and shotgun metagenomics capture compositional changes at the genus and species level, yet these data also highlight the need for strain-level resolution. Strains within the same species differ markedly in ecological fitness, mobile genetic element (MGE) content, and capacity to acquire or retain ARGs, factors that may influence whether disturbances resolve rapidly or leave long-term signatures[88,89]. Strain-resolved analyses would therefore allow us to distinguish complementary mechanisms underlying microbiota shifts and ARG acquisition: temporary and/or persistent dominance of exogenous strains introduced during travel versus in situ horizontal transfer of ARG-bearing MGEs into resident lineages[89].

Strain tracking would also enhance geographical interpretation. Several travel studies, including our own (**Chapter 5**), identify South and Southeast

Asia as hotspots for ARG acquisition[52,62,90]. Integrating resistome shifts with strain or sequence-type information (for example, *E. coli* phylogroups) can reveal whether region-specific lineages are being imported or whether local environmental pressures primarily select for function while the genomic background remains host-native[91,92]. Likewise, strain-level data can clarify how recovery after travel occurs, showing whether travel-acquired strains are replaced by the original resident community or whether resident strains persisted but temporarily carried plasmids that were later lost. These considerations motivate the incorporation of long-read or hybrid metagenomics to resolve plasmid–host linkages and targeted high-throughput culturing (culturomics) to connect genotype with phenotype[89,93]. Deep or hybrid sequencing approaches are especially valuable for characterizing low-abundance but ecologically important taxa[94]. Moving forward, adopting multi-omic frameworks that combine microbiome profiling with host transcriptomics, metabolomics, proteomics, and immune signatures could provide deeper insight into microbiome trajectories. Such integrative approaches have already improved IBS subtype classification[26,95] and may be particularly informative in PI-IBS. For instance, combining microbiome analysis with proteolytic activity offered a mechanistic basis for distinguishing high- and low-proteolytic PI-IBS phenotypes[28]. In addition, examining transcriptomic and metabolomic correlations with altered intestinal permeability[96], provide promising paths to disentangle how microbial activity and host physiology interact to shape vulnerability to, and recovery from, post-infection outcomes. At the same time, these advances must be approached with caution. Multi-omics increases analytical complexity and the potential for over-interpretation, and the field must balance the search for reproducible, clinically actionable markers with the recognition that individual trajectories are highly context-dependent. This is underscored by the fact that taxa implicated in PI-IBS in our analyses, such as *E. lenta*, *R. gnavus*, and *B. fragilis*, are also common in healthy individuals[97-99]. In particular, *E. lenta* exhibits considerable strain-to-strain variation in metabolic and immunomodulatory capacity, suggesting that only certain lineages may drive disease-related phenotypes[100] In addition it is also possible that organisms like *E. lenta* only become harmful under specific host conditions, such as heightened psychological stress or immune activation. These considerations underscore the need to move beyond broad microbiome metrics toward integrative frameworks that incorporate ecological, functional, and host-related dimensions.

Including other microbes

While this thesis focused primarily on bacterial community dynamics, the gut microbiome is a multispecies ecosystem that includes viruses, fungi, archaea, and protozoa such as *Blastocystis*. These domains are increasingly recognized as active participants in shaping host–microbe interactions. For instance, each individual also has a persistent personal gut virome [101] and virome profiles have been linked to stress-induced changes in behaviour and immune function in animal models [102]. *Blastocystis* has been linked with improved cardio metabolic profiles [103] and has also been shown to alter cognitive function in mice [104]. Thus, these additional microbes may also contribute to PI-IBS development and warrant careful consideration in future research.

Role of machine learning and other upcoming tools

Advances in computational modelling, particularly in machine learning (ML) and artificial intelligence (AI), have led to their increasing application to omics datasets across a wide range of conditions associated with microbiome. These include chronic disorders and diseases such as IBD[105], IBS[106], Type 2 diabetes[107], Parkinson's disease[108],colorectal cancer[109,110], as well as studies addressing multiple diseases simultaneously[27,111]. Overall, these examples demonstrate the potential of ML in clinical settings, from discovering microbial biomarkers for disease risk or health classification to predicting responses to therapies or diets and tailoring microbiome-targeted interventions. Machine learning was also applied in the present thesis to PI-IBS (**Chapters 3 and 4**), where microbiota and questionnaire data were successfully used for risk classification and the identification of microbiota-based predictors. ML might also be useful to predict AMR acquisition or persistence (**Chapter 5 and 6**) as previous study on whole-genome sequenced data were able to identify mutations, gene regions or bacterial phenotypes associated with AMR[112-114].

In addition, there is considerable potential to enhance metadata collection, particularly with advances in wearable devices and mobile applications. For example, tracking sleep patterns during travel[115,116] and incorporating geolocation data could be linked to the timing of initial gastroenteritis symptoms and or the acquisition and dissemination of AMR[117,118]. Collecting such rich metadata offer clear advantages, as demonstrated by Shmatko et al., who showed that patterns of disease progression can be predicted from large-scale health record datasets using their AI model (Delphi-2M) trained on data from approximately 0.4 million UK Biobank participants[119]. However, enthusiasm for ML and AI applications, as well as for the automated collection of large and diverse datasets, must be balanced with caution. The credibility of inferences, particularly in microbiome research, depends on rigorous validation, transparency, and reproducibility[120], alongside robust evidence of safety and clear clinical utility. Such utility should include demonstrable improvements over current standards of care, for example in diagnostic accuracy or patient outcomes[121]. Moreover, strict (microbiome) data protection and privacy standards are essential to prevent misuse of sensitive health information[122]. Despite rapid progress, widely accepted guidelines for assessing the performance, safety, and clinical readiness of ML and AI models in healthcare remain limited[120]. In the context of travellers, future studies should assess whether predictive models maintain their performance across diverse, real-world populations and evaluate how they can be integrated into pre-travel counselling or post-travel follow-up while fully respecting traveller and patient privacy and data-protection requirements.

Therapeutics for modulating the traveller microbiome

The potential for microbiome-directed therapies in reducing PI-IBS risk deserves further exploration. Probiotics, prebiotics, or even targeted microbiota-modulating strategies could help stabilise the microbiome during travel, reduce susceptibility to infection, and improve recovery after perturbation. Faecal microbiota transplantation (FMT), highly successful in recurrent C. difficile infection[123], has yielded mixed results in IBS[124-127]. While evidence remains preliminary, our findings (**Chapter 4 and 6**) suggest that strategies aimed at preserving microbial resilience could provide benefits for both individual travellers and public health. Interventional trials in the travel setting offer a logical next step to determine whether prophylactic or therapeutic modulation of the microbiome can enhance resilience, reduce TD incidence and thus indirectly PI-IBS development, thereby moving the field from association to actionable intervention. Mechanistic insights also highlight promising therapeutic avenues. Culp et al. systematically mapped interactions between approximately 150 dietary xenobiotics and the gut microbiome, demonstrating that microbial metabolism of these compounds, including resveratrol, drives community-specific and interindividual variation in microbiome responses to diet[128]. Microbial metabolites such as tryptamine can influence colonic fluid secretion and thereby alleviate slow gastrointestinal transit[129], while host–microbe signalling through receptors such as the aryl hydrocarbon receptor (AhR) modulates neuronal and epithelial physiology[130,131]. These findings raise the possibility of targeted and personal interventions that steer host–microbe crosstalk prior, during or after travel, supporting prevention of microbiome perturbation or promoting its restoration following disturbance.

Looking forward

This thesis illustrates the permeability of the interface between the environment and the human gut. Just as souvenirs carried home are tangible reminders of travel, ARGs represent invisible microbial souvenirs (**Chapters 5 and 6**) which are acquired through transient or persistent colonisation with AMR bacteria and contribute to the global dissemination of AMR[132]. Meanwhile, post-travel complications like PI-IBS are long-lasting souvenirs that are difficult to dispose of, manifesting as persistent visceral symptoms and altered bowel habits as highlighted in **Chapter 3** and **Chapter 4**. We showed that travel acts not as a passive event but has the potential to act as a catalyst for ecological and evolutionary changes within the gut. This thesis reminds us that the gut is not only a personal organ but also a crossroads of ecological and societal processes. The gut, in this sense, becomes a site where microbes compete, adapt, and exchange traits under the selective pressures imposed by travel including infection, medicine use, and diet effects as well as potential effects of circadian rhythm disruptions (**Chapter 2)**. A unified microbiome exposure and disruption framework may explain why travel can yield chronic symptoms in some and asymptomatic AMR carriage in others, with overlapping host and behavioural risk profiles. Building on this, the next phase of research should focus on methodological innovations, embrace multi-omic and computational tools, and move towards interventional and policy applications. By doing so, the field can refine its understanding of travel-associated risks and develop strategies to mitigate them at both the individual and societal level.

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CHAPTER 8

Impact paragraph

According to the United Nations World Tourism Organization, international tourism recovered to nearly pre-pandemic levels in 2024, with around 1.4 billion people travelling across borders that year[1]. This resurgence demonstrates the world's renewed appetite for connection and underscores how mobile and globally interlinked human life has become. Every day, millions of travellers move between continents for work, study, and leisure. However, while these journeys connect cultures and economies, they also connect the microorganisms living in our guts that play essential roles in digestion, immunity, and overall health. This hidden ecosystem is as personal as a fingerprint and, although generally stable, it is highly responsive to new and unfamiliar environmental exposures. The research presented in this thesis aimed to elucidate how international travel influences this gut ecosystem, focusing on the development of post-infectious irritable bowel syndrome (PI-IBS) and the acquisition and persistence of antimicrobial resistance genes (ARGs). By combining longitudinal microbiome profiling, metagenomic sequencing, and clinical follow-up data, the thesis provides novel insights into how travel-associated exposures can transiently or permanently alter gut microbial ecology and contribute to ARG acquisition or PI-IBS development. The findings highlight key microbial and environmental determinants that shape travel-related health outcomes. This section describes how these findings contribute to scientific progress and its societal relevance and impact.

Scientific relevance and impact

From a scientific perspective this research makes at least two key contributions. First, by studying travellers before, during, and after their journeys, our research was the first to study risk factors as well as microbiota perturbations in new onset PI-IBS. This is a major step towards disentangling causes from consequences and epiphenomena in the aetiology of IBS. We amongst others showed that disruptions in gut microbiota diversity and resilience may increase the risk of developing PI-IBS. The observations that these differences in gut microbial diversity and composition were already evident prior to travel suggest a biological predisposition that could one day guide early detection or preventive care. This opens the door to personalized strategies such as targeted prebiotics, probiotics, or dietary adjustments to maintain or restore microbiota balance following travel or infection, but also microbiome monitoring for high-risk individuals. This approach represents a step toward personalized, microbiome-informed medicine, where maintaining a healthy gut ecosystem becomes a key aspect of preventing chronic disorders. It parallels the concept of vaccinations for preventing infectious diseases when traveling to specific regions, highlighting proactive, tailored health strategies. At the same time, several specific bacterial species were found to be consistently elevated or lower in people prior to PI-IBS development. Future research on these bacterial taxa may uncover mechanistic understanding to better understand the pathology of IBS, helping to validate it as a distinct disease and enhance our ability to understand and address the patient experience effectively.

Second, this thesis reveals how quickly antibiotic resistance genes can be acquired during travel, sometimes within days, and how these genes can persist long after returning home. These findings highlight the need to better understand the routes of exposure, including contaminated food and water, and shared public facilities. Regarding the latter, it is plausible that acquisition may begin as early as at airports, where travellers from every corner of the globe are exposed to diverse microbial communities, providing a critical early point for intervention and surveillance.

Societal relevance and impact

Combating and monitoring AMR spread

The outcomes of this research are highly relevant in the context of global travel, healthcare, and antimicrobial stewardship, as increasing human mobility creates opportunities new opportunities for the worldwide spread of AMR, positioning travellers both as sentinels and potential vectors of AMR. By identifying risk factors and microbial signatures associated with AMR acquisition and persistence, this research supports evidence-based policies to minimize the dissemination of resistant bacteria across borders. These insights can inform travel medicine practices, such as pre-travel counselling, hygiene recommendations, and post-travel screening for high-risk groups.

Currently, infection-prevention protocols in Dutch and European healthcare settings primarily screen patients for multidrug-resistant organisms (MDROs) only after recent hospitalization abroad—regardless of whether that occurred in neighbouring countries such as Germany or in high-risk regions such as South or Southeast Asia. However, emerging evidence, including the findings of this thesis, indicates that individuals who travel to high-risk regions (e.g., Asia or Africa) without hospital exposure also carry ARGs upon return.

The Dutch national partnership responsible for developing, harmonizing, and maintaining infection prevention guidelines (Samenwerkingsverband Richtlijnen Infectiepreventie) already recognizes this elevated risk in its guidance, advising healthcare facilities to remain alert to possible extended-spectrum β-lactamase–producing Enterobacterales (ESBL-E), carbapenemase-producing Enterobacterales (CPE), or carbapenem-resistant Enterobacterales (CRE) carriage among travellers returning from these regions[2]. Yet, this group has not been formally included in standard risk assessments for routine screening. The findings of this thesis provide empirical evidence to support reconsideration of these criteria, suggesting that travel history should be evaluated as a potential risk factor for AMR acquisition. Furthermore, we demonstrate that travellers can carry AMR for several months after returning, highlighting the need to consider extended post-travel monitoring in addition to recent travel history. This prolonged carriage increases the risk of introducing AMR into hospital and healthcare settings, even months after travel. Incorporating these insights could enable more targeted and efficient screening strategies after travel, at hospital admission and during follow-up, thereby reducing the likelihood of unrecognized introduction and spread of resistant organisms within healthcare environments.

Beyond infection prevention, this research highlights a broader public health consideration. Current screening practices in the Netherlands differentiate between returning travellers and groups such as asylum seekers [2], despite limited evidence supporting higher AMR carriage among the latter. The findings of this thesis emphasize that international travel plays a major role in the global dissemination of AMR, and other experts have similarly argued that intercontinental travel contributes more to microbial exchange than migration [3]. Recognizing AMR dissemination as an outcome of growing global mobility underscores the need for fair and evidence-based control measures that address modern travel realities without reinforcing stigma.

Increased awareness of (PI-)IBS

At the same time, our findings help raise awareness of irritable bowel syndrome (IBS) as a common and often misunderstood condition, emphasizing that travel can contribute to its onset. By collaborating with IBS advocacy organizations and sharing our research progress to patients, healthcare providers and dieticians, we aimed to strengthen the exchange of knowledge and promote wider understanding of this disorder [4]. Many individuals living with IBS continue to face misconceptions and stigmatization due to the absence of structural abnormalities and measurable pathophysiological mechanisms as well as the variable nature of their symptoms[5]. Our research brings us one step closer to unravelling the biological underpinnings of IBS, thus laying the groundwork for developing predictive and diagnostic tools that can improve patient outcomes. By identifying measurable biological causes, our work contributes to shifting the perception of IBS from a vague or psychosomatic disorder toward a recognized disease with clear foundations. This contributes to validating patients' experiences and guide more effective care. Moreover, the link between enteric infections and PI-IBS provides valuable knowledge for improving patient care and early intervention strategies. The type of pathogen responsible for traveller's diarrhoea is a key factor in the development of PI-IBS. Gaining a deeper understanding of how acute infections and microbiota disruptions lead to chronic gastrointestinal symptoms can aid clinicians in identifying individuals at higher risk. Our research contributes to this body of knowledge, and further investigation in this area will pave the way for tailored management strategies that not only prevent long-term health complications but also support microbiome recovery, ultimately aiming to improve patients' quality of life and reduce the healthcare burden.

Towards holistic understanding

In addition, our work reshapes our view of global health and personal responsibility. Many people still think of their gut bacteria as something static or unrelated to daily life. Yet this research shows that our microbial world is continuously shaped by what we eat, where we go, and the environments we encounter. Our finding that our gut microbiota is involved in the development of PI-IBS and acquisition of AMR emphasizes that travel health is not only about vaccinations and hygiene but also about protecting and restoring the gut ecosystem. Educating travellers, clinicians, and policymakers about these links can foster a more holistic understanding of health that extends beyond humans to include our microbial partners.

Furthermore, this research contributes to a growing global dialogue on the deep interconnectedness of human, animal, and environmental health, e.g. the “One-Health concept. Our finding that shellfish consumption increases the risk of ARG acquisition reinforces this connection. By mapping how travel acts as a bridge between various microbial environments, this work supports a more integrated approach to tackling AMR and maintaining microbial diversity at a planetary scale. Ultimately, this research reminds us that while we may cross borders, so do our microbes. Recognizing this interconnectedness is essential to safeguarding both human health and planetary health.

Dissemination

Scientific community: This thesis contributes valuable datasets and insights to ongoing research exploring how the gut microbiota influences PI-IBS development and antimicrobial resistance gene acquisition. These findings have been shared with national and international research communities at conferences such as the Dutch Microbiome Day and International Human Microbiome Consortium (IHMC) Congress, supporting further collaboration and discovery in this field. In adherence to the FAIR principles[6], all sequencing data are being or will be deposited in publicly accessible repositories in a manner that ensures they are findable, accessible, and interoperable, facilitating reuse by other researchers Also the codes for the data-analysis are accessible or will be made accessible via the GitHub of the department of Medical Microbiology, Infectious Diseases and Infection Prevention.

Travellers, patients, and clinicians: Our research results are relevant to traveller health concerns and clinicians dealing with travellers. It lays an important groundwork for future research into IBS pathology as well as potential for developing strategies to target at risk groups to prevent IBs after gastroenteritis. Our team communicates with the Dutch Digestive Health Fund (MDL Fonds), PDSB (Irritable Bowel Syndrome Advocacy Organization), and medical professionals through podcast and newsletters to share our findings and raise awareness of our research [4,7]. I discussed the potential of gut microbial profiling for the diagnosis and prediction of PI-IBS in the medical podcast *Door het oog van de scoop* [8]. To support clinicians and fellow researchers in staying informed, we have published or submitted our findings in open-access journals. We have also presented our work at several national symposia, including the Scientific Spring Meeting organized by the Nederlandse Vereniging voor Medische Microbiologie (NVMM) and the Koninklijke Nederlandse Vereniging voor Microbiologie (KNVM), the Havensymposium on travel medicine, the Maastricht Symposium on Migrants and Travel Medicine, as well as the clinically oriented Digestive Disease Days (DDD) of the Dutch Association for Gastroenterology.

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CHAPTER 9

English summary

Nederlandse samenvatting

Summary

Globalisation has increased intercontinental travel, exposing individuals to new environments, diets, and microbial communities that can disrupt the gut microbiome, promote the acquisition of antimicrobial resistance (AMR) genes, and contribute to post-infectious complications. This thesis aimed to characterise the impact of intercontinental travel on the gut microbiota and AMR gene acquisition, and to investigate the role of the travellers' gut microbiota in post-infection irritable bowel syndrome (PI-IBS) using prospective cohort data and longitudinal microbiota analyses.

Chapter 1 introduced the health implications of modern travel, highlighting how increased global mobility exposes travellers to risks such as travellers' diarrhoea (TD), PI-IBS development and AMR acquisition. The gut microbiota acts as a central interface linking these processes. Finally, the aims and scope of the thesis were outlined in this chapter.

In **Chapter 2**, we synthesised current knowledge on gut microbiome and antimicrobial resistance (AMR) gene dynamics in travellers. This review highlights how intercontinental travel influences microbial diversity, composition, and function, while facilitating the acquisition and global dissemination of AMR genes. Travellers are presented as a valuable group for studying environmental influences on the gut microbiome and resistome. We further discussed potential mechanisms underlying microbiome disruption and recovery following travel, including pathogen exposure, dietary changes and medicine use. The chapter identified knowledge gaps and outlines methodological considerations necessary to better understand microbiome perturbations and AMR acquisition and spread in travellers.

In **Chapter 3**, we investigated the incidence and predictors of PI-IBS following intercontinental travel using data from the prospective COMBAT (Carriage of Multiresistant Bacteria After Travel) cohort study. Among 539 travellers who experienced TD and had no prior IBS, 65 developed PI-IBS, corresponding to an incidence of 12.1%. The highest incidence was observed among travellers to Southern Asia. Several independent predictors of PI-IBS were identified, including stomach cramps, chronic illness, nausea, weight loss, antibiotic use, antacid use, vegan or vegetarian diet, and poorer self-rated health. In addition to traditional statistical approaches, machine learning models were applied to predict PI-IBS risk, with neural networks showing good predictive performance.

In **Chapter 4**, we further explored the relationship between intercontinental travel and the gut microbiota using longitudinal microbiota data from 639 participants of the COMBAT cohort, comprising faecal samples collected before and after travel. Travel was associated with transient reductions in microbial diversity and changes in community composition, strongly influenced by antibiotic use, enteric symptoms, and behavioural exposures. Case–control analyses revealed that individuals who later developed PI-IBS exhibited lower microbial diversity before and after travel and had distinct microbial communities enriched in specific taxa including *Bacteroides fragilis*, *Eggerthella lenta*, and *Ruminococcus gnavus*. Machine learning models based on microbiota profiles were able to predict PI-IBS and were validated in an independent IBS cohort (Maastricht IBS cohort). Together, the results provide evidence that microbiota composition and resilience may influence susceptibility to developing PI-IBS.

In **Chapter 5**, we examined the acquisition of antimicrobial resistance genes among 637 Dutch intercontinental travellers participating in the COMBAT study. Using quantitative Polymerase Chain Reaction (qPCR)-based targeted approach, we assessed the presence of genes associated with resistance to extended-spectrum beta-lactams, quinolones, colistin, and aminoglycosides before and after travel. Risk factors for acquisition varied between AMR genes but commonly included destination region, antibiotic use, chronic disease, and dietary exposures such as shellfish consumption.

To capture short-term microbial and AMR dynamics during travel, **Chapter 6** showed application of a high-frequency sampling approach in a smaller cohort of eleven Dutch travellers to Asia. Participants self-collected faecal samples before, during, and after travel. Microbiota composition was assessed using 16S rRNA gene sequencing and beta-lactams and quinolones resistance genes were quantified using targeted quantitative-PCR. Longitudinal analyses revealed inter- and intra-individual variation in microbiota composition, with rapid shifts occurring within the first days of travel. The first days of travel were characterised by an increase in Enterobacterales and a decrease in commensal taxa, coinciding with the acquisition of AMR genes.

Finally in **Chapter 7**, the findings of this thesis are integrated and discussed within the broader context of microbiome research, travel medicine, and antimicrobial resistance. The chapter reflects on how intercontinental travel can act as a catalyst for ecological changes in the gut microbiome, facilitating both transient and persistent microbial alterations. It highlights how travellers may acquire “microbial souvenirs” in the form of AMR genes, while also experiencing long-term health consequences such as PI-IBS after travel. The chapter further discusses methodological strengths and public health and clinical implications and outlines future research directions aimed at mitigating and better understanding travel-associated microbiome disruption.

Samenvatting

Door globalisering is intercontinentaal reizen sterk toegenomen waardoor mensen worden blootgesteld aan nieuwe omgevingen, voedingspatronen en microbiële gemeenschappen. Deze blootstellingen kunnen het darmmicrobioom verstoren, de acquisitie van genen voor antimicrobiële resistentie (AMR) bevorderen en bijdragen aan postinfectieuze complicaties. Dit proefschrift had tot doel de impact van intercontinentaal reizen op de darmmicrobiota en de verwerving van AMR-genen te karakteriseren, evenals de rol van de darmmicrobiota bij het ontstaan van postinfectieus prikkelbare darmsyndroom (PI-PDS) te onderzoeken. Daarbij werd er gebruikgemaakt van prospectieve cohortgegevens en longitudinale microbiota-analyses.

Hoofdstuk 1 introduceert de gezondheidsimplicaties van reizen en benadrukt hoe toegenomen wereldwijde mobiliteit reizigers blootstelt aan risico's zoals reizigersdiarree, de ontwikkeling van PI-IBS en de verkrijging van AMR. De darmmicrobiota treedt hierbij op als een centrale schakel. Tot slot worden de doelstellingen en het bereik van het proefschrift uiteengezet.

In **hoofdstuk 2** wordt de huidige kennis over de dynamiek van het darmmicrobioom en antimicrobiële resistentiegenen (AMR) bij reizigers samengevat. Dit overzicht laat zien hoe intercontinentaal reizen de microbiële diversiteit, samenstelling en functie beïnvloedt en tegelijkertijd de verwerving en wereldwijde verspreiding van AMR-genen faciliteert. Reizigers vormen een interessante populatie voor het bestuderen van omgevingsinvloeden op het darmmicrobioom en het resistoom. Daarnaast worden mogelijke mechanismen besproken die ten grondslag liggen aan de verstoring en herstel van het microbioom na reizen zoals blootstelling aan pathogenen, veranderingen in dieet en medicijngebruik. Het hoofdstuk identificeert ontbrekende kennis en beschrijft methodologische overwegingen die nodig zijn om deze processen beter te begrijpen.

In **hoofdstuk 3** onderzochten we de incidentie en de voorspellende factoren van PI-IBS na intercontinentaal reizen met gegevens uit de prospectieve COMBAT-cohortstudie (Carriage of Multiresistant Bacteria After Travel). Van de 539 reizigers met reizigersdiarree en zonder voorgeschiedenis van PDS, ontwikkelden 65 personen PI-PDS, wat neerkomt op een incidentie van 12,1%. De hoogste incidentie werd waargenomen bij reizigers naar Zuid-Azië. Verschillende onafhankelijke voorspellende factoren werden geïdentificeerd, waaronder maagkrampen, chronische ziekte, misselijkheid, gewichtsverlies, antibioticagebruik, het gebruik van maagzuurremmers, een veganistisch of vegetarisch dieet en een slechtere zelfgerapporteerde gezondheid. Naast traditionele statistische methoden werden ook computationele leermodellen (machine learning) toegepast, waarbij de "neural network" methode goede voorspellende prestaties lieten zien.

Hoofdstuk 4 onderzoekt verder de relatie tussen intercontinentaal reizen en de darmmicrobiota met behulp van longitudinale gegevens van 639 deelnemers aan het COMBAT-cohort, gebaseerd op fecale monsters verzameld vóór en na de reis. Reizen ging gepaard met tijdelijke afnames in microbiële diversiteit en veranderingen in de samenstelling van de microbiële gemeenschap, sterk beïnvloed door antibioticagebruik, darmklachten en gedragsfactoren. Case-control-analyses toonden aan dat personen die later PI-PDS ontwikkelden, zowel vóór als na de reis, een lagere microbiële diversiteit hadden en specifieke microbiële profielen vertoonden. Dit werd gekenmerkt door toename van taxa zoals *Bacteroides fragilis*, *Eggerthella lenta* en *Ruminococcus gnavus*. Machine learning-modellen gebaseerd op microbiotaprofielen konden PI-PDS voorspellen en werden gevalideerd in een onafhankelijk PDS-cohort (Maastricht PDS-cohort). Deze resultaten suggereren dat de samenstelling en veerkracht van de microbiota van invloed zijn op de vatbaarheid voor PI-PDS.

In **hoofdstuk 5** wordt de acquisitie van antimicrobiële resistentiegenen onderzocht bij 637 Nederlandse intercontinentale reizigers uit de COMBAT-studie. Met behulp van een op kwantitatieve PCR (qPCR) gebaseerde gerichte aanpak werd de aanwezigheid van genen geassocieerd met resistentie tegen breedspectrum-bètalactamantibiotica, chinolonen, colistine en aminoglycosiden vóór en na de reis bepaald. De risicofactoren voor verwerving verschilden per AMR-gen, maar omvatten doorgaans de bestemmingsregio, antibioticagebruik, chronische ziekte en blootstelling via voeding, zoals consumptie van schaaldieren.

Hoofdstuk 6 richt zich op de korte termijn dynamiek van het microbioom en de ontwikkeling van AMR tijdens reizen, door middel van frequente bemonstering bij een kleinere groep van 11 Nederlandse reizigers naar Azië. De deelnemers verzamelden zelf fecesmonsters vóór, tijdens en na de reis. De microbiota samenstelling werd geanalyseerd met 16S ribosomaal-RNA-gen sequencing en resistentiegenen tegen bètalactamantibiotica en chinolonen werden gekwantificeerd via qPCR. Longitudinale analyses toonden zowel inter- als intra-individuele variatie, met zichtbaar snelle veranderingen in de eerste dagen van de reis. Deze periode werd gekenmerkt door een toename van Enterobacterales en een afname van commensale taxa, gelijktijdig met de verkrijging van AMR-genen.

Tot slot worden in **hoofdstuk 7** de bevindingen van dit proefschrift verenigd en geplaatst in de bredere context van microbioom onderzoek, reisgeneeskunde en antimicrobiële resistentie. Het hoofdstuk beschrijft hoe intercontinentaal reizen processen in gang kan zetten voor ecologische veranderingen in het darmmicrobioom, met zowel tijdelijke als blijvende effecten. Reizigers kunnen "microbiële souvenirs" meenemen in de vorm van AMR-genen en tegelijkertijd risico lopen op langdurige gezondheidsklachten zoals PI-PDS. Daarnaast worden de methodologische sterke punten, implicaties voor de volksgezondheid en klinische praktijk besproken, evenals toekomstige onderzoekslijnen gericht op het beter begrijpen en beperken van reisgerelateerde verstoringen van het microbioom.

ADDENDUM

Acknowledgements

List of publications

About the author

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Now that my PhD period comes to an end, I've had some time to reflect. This thesis is not just my work, but really a culmination of the efforts of many people around me. I feel like I've been part of a big journey, contributing my part and hopefully passing the baton on to those who follow. Because of that, I feel both grateful and a little sad to move on from this group. Over the years, I've met incredible people who became my mentors, colleagues and friends. We shared challenges, supported each other through ups and downs, and grew together both professionally and personally. My journey began small, but it quickly filled with many colourful people. It's impossible to capture all my gratitude and everything I feel here, but I will try.

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1. **Chan, J.**, van Best, N., Arcilla, M., van Hattem, J., Melles, D.C., de Jong, M.D., Schultsz, C., van Genderen, P.J., Bootsma, M.C., and Goorhuis, A. (2024). Antimicrobial resistance genes acquisition among Dutch intercontinental travellers: a prospective multicentre study. Journal of Travel Medicine *31*, taae131. https://doi.org/10.1093/jtm/taae131.
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About the author

Jiyang Chan was born on the 19th of July 1993, in Schiedam, the Netherlands, and grew up in Maassluis. He obtained his Voorbereidend Wetenschappelijk Onderwijs (VWO) degree from SG Spieringshoek in Schiedam in 2012. He subsequently pursued a Bachelor of Science in Biology at Leiden University, where he conducted research on genome editing in Arabidopsis thaliana using CRISPR-Cas9 technology.

He continued with the Master's programme in Bio-Pharmaceutical Sciences at Leiden University, specializing in Drug Discovery and Safety, where he completed an internship focused on computational modelling of drug-induced liver injury (DILI). During this period, he developed a growing interest in epidemiology and population-level health processes, which motivated his transition to the Research Master in Health Sciences at Maastricht University in 2019.

As part of this programme, he undertook a nine-month internship under the supervision of Dr. John Penders in the Department of Medical Microbiology, Infectious Diseases and Infection Prevention. His research focused on the acquisition of antimicrobial resistance (AMR) genes and the development of post-infectious irritable bowel syndrome in relation to gut microbiota dynamics in travellers.

After graduating in 2021, he began his PhD in the same department under the supervision of Dr. John Penders and Dr. Niels van Best. His doctoral research built upon and expanded this work, ultimately leading to this thesis.